RELIABLE DIAGNOSTIC TECHNIQUES FOR CANINE DIABETES WITH HISTOPATHOLOGICAL EFFECTS OF ORGANS

Sonnet Poddar

Roll No. 0116/02

Registration No. 00283

Session: 2016 – 2017

A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Anatomy

Department of Anatomy and Histology

Faculty of Veterinary Medicine

Chittagong Veterinary and Animal Sciences University

Chittagong – 4225, Bangladesh

SEPTEMBER 2018

RELIABLE DIAGNOSTIC TECHNIQUES FOR CANINE DIABETES WITH HISTOPATHOLOGICAL EFFECTS OF ORGANS

Sonnet Poddar

Roll No: 0116/02

Registration No: 00283

Session: 2016 – 2017

……..

(Prof. Dr. Mohammad Mejbah Uddin)

Supervisor

Department of Anatomy and Histology

Chittagong Veterinary and Animal Sciences University, Khulshi – 4225, Chittagong

……..

(Prof. Dr. Mohammad Mejbah Uddin)

Chairman of the examination committee

Department of Anatomy and Histology

Chittagong Veterinary and Animal Sciences University, Khulshi – 4225, Chittagong

Department of Anatomy and Histology

Faculty of Veterinary Medicine

Chittagong Veterinary and Animal Sciences University

Chittagong – 4225, Bangladesh

September, 2018

Acknowledgements

The author wishes to acknowledge the immeasurable grace and profound kindness of Almighty "GOD" the supreme authority and supreme ruler of universe, who empowers the author to complete the research work and thesis successfully for the degree of Master of Science (MS) in Anatomy.

The author gratefully express his heartiest appreciation, deepest sense of gratitude and best regards to his research supervisor, honorable **Professor Dr. Mohammad Mejbah Uddin,** Head, Department of Anatomy and Histology, Faculty of Veterinary Medicine of Chittagong Veterinary and Animal Sciences University for his advice, encouragement, constructive criticism, scholastic supervision and intellectual guidance throughout this research.

The author wishes to express his deep sense of gratitude, gratefulness and heartfelt thanks to **Professor Dr. Lutfur Rahman, Professor Dr. Kh. Nurul Islam, Professor Dr. A. S. M. Lutful Ahasan, Dr. A. S. M. Golam Kibria, Associate professor and DR. Abdullah Al Faruq, Lecturer** at the Department of Anatomy and Histology, Faculty of Veterinary Medicine of Chittagong Veterinary and Animal Sciences University for their factual advice, kind cooperation and continuous encouragement during the study.

NST and **UGC** is thankfully acknowledged for providing fund to post-graduate research grant and making a platform to conduct this research work.

The author would like to special thanks to **DR. Shahriar Hasan Sohel**, MS student at Department of anatomy and histology, **Md. Bayazid Hasan Dhali,** the laboratory technician, **Md. Alauddin,** staffs and personnel of the department of Anatomy and Histology, Chittagong Veterinary and Animal Sciences University for their assistance in conducting laboratory examination of samples.

The author is grateful to his friends and well-wishers for their kind co-operation during the research work

Finally the author would like to give heartiest gratitude to his parents, brothers and relatives for their great sacrifices, infinite patience, spontaneous blessings, continuous encouragement and dedicated effort for a period of years.

The author,

September, 2018

Table of contents

Contents	Page No
Acknowledgements	**i**
Table of contents	**ii - iii**
List of table	**iv**
List of figures	**v**
List of abbreviation	**vi**
Abstract	**vii**
Chapter - 1: Introduction	**01 – 02**
1.1 General introduction with background	01
1.2 Objectives	02
Chapter -2: Review of literature	**03 – 08**
2.1 Canine diabetes and its incidence	03
2.2 Etiology of canine diabetes	04
2.3 Clinical signs of canine diabetes	04 – 05
2.4 Diagnosis methods of canine diabetes	05 – 06
2.5 Biochemical changes of blood in canine diabetes	06 – 07
2.6 Histopathological effect of canine diabetes on different organs	07 – 08
Chapter -3: Materials and Methods	**09 – 14**
3.1 Study area and study population	09
3.2 Experimental design	09
3.3 Determination of the disease	09 – 11
3.3.1 Fasting glucose test in blood by PBGM device	10
3.3.2 Bio-chemical analysis of blood, urine of dogs for determination of diabetes mellitus	10 – 11
3.3.3 Oral glucose tolerance test	11
3.4 Collection of histopathological specimens	11
3.5 Histopathological study of the specimens	11 – 12
3.5.1 Preparation of permanent slide	11 – 12
3.5.2 Study of histopathological effect on different organs	12
3.6 Statistical analysis of data	12
3.7 Photography	12

Contents	Page No
Chapter -4: Results	**15 – 26**
4.1 Blood glucose evaluation in dog	15
4.2 Determination of glucose by laboratory qualitative testes	15 – 16
4.3 Hematological profile evaluation	16
4.4 Serum biochemical profile evaluation	16 – 18
4.5 Oral glucose tolerance test evaluation	18 – 19
4.6 Reliable diagnostic technique	19 – 20
4.7 Histopathological effect of different organs	20 – 21
Chapter -5: Discussion	**27 – 31**
5.1 Clinical signs and prevalence of diabetic	27
5.2 Blood glucose in diabetic dog	27 – 28
5.3 Hematological profile of diabetic dog	28 – 29
5.4 Serum biochemical profile of diabetic dog	29 – 30
5.5 Reliable diagnostic technique	30 – 31
5.6 Histopathological effects of different organs of diabetic dog	31
Chapter - 6: Conclusion	**32**
Chapter – 7: Recommendations and future perspectives	**33**
References	**34 – 39**
Appendix	**40 – 41**
Biography	**42**

List of Tables

Table No	Title	Page No
Table 1	Blood glucose level evaluation in dog by portable blood glucose measurement device	15
Table 2	Laboratory findings	15
Table 3	Hematological profile evaluation	16
Table 4	Serum biochemical profile evaluation	17
Table 5	Oral glucose tolerance test for evaluation of experimental dogs	19
Table 6	Sensitivity and specificity of different test for diagnosis of diabetes in dog	20

List of Figures

Figure No	Title	Page No
Figure 1	Study area, SQTVH, CVASU, Chittagong metropolitan area	09
Figure 2	Diagnostic tools for the detection of diabetes in Dog	13
Figure 3	Dissection, collection and preservation of different tissue from diabetic and control dog	14
Figure 4	a. Hematological profile of diabetic and control dogs and b. Biochemical profile of diabetic and control dogs	18
Figure 5	Histopathological effect of pancreas of diabetic dog compared with normal control dog in hematoxylin and eosin staining (4X and 10X).	22
Figure 6	Histopathological effect of liver of a diabetic dog compared with normal control dog in hematoxylin and eosin staining (4X and 10X).	23
Figure 7	Histopathological effect of kidney of diabetic dog compared with normal control dog in hematoxylin and eosin staining (4X and 10X).	24
Figure 8	Histopathological effect of small intestine (duodenum; jejunum) of diabetic dog compared with normal control dog in hematoxylin and eosin staining (4X)	25
Figure 9	Histopathological effect of large intestine (Caecum; colon) of diabetic dog in hematoxylin and eosin staining (4X)	26

List of Abbreviations

Abbreviation and Symbol	Elaboration
CVASU	Chittagong Veterinary and Animal Sciences University
SAQTVH	Shahedul Alam Quadary Teaching Veterinary Hospital
DM	Diabetes Mellitus
BG	Blood Glucose
CBC	Complete Blood Count
UPC	Urine Protein Creatinine ratio
HE	Hematoxylin and Eosin
T_4	Thyroxin
EDTA	Ethylenediamine Tetraacetic Acid
PCV	Packed Cell Volume
TEC	Total Leucocyte Count
TEC	Total Erythrocyte Count
Hb	Hemoglobin
WBC	White Blood Cell Count
ALT	Alanine Transaminase
ANOVA	Analysis of Variance
SN	Sensitivity
SP	Specificity
PPV	Positive Predict Value
NPV	Negative Predict Value.
PBGM	Portable Blood Glucose Measurement Device
HbA1c%	Glycated Haemoglobin
APAL	Atypical Pancreatic Acinar Lobule
TPAL	Typical Pancreatic Acinar Lobule
FD	Fatty Deposition
DPI	Degeneration of Pancreatic Islet,
Fb	Fibrosis
Nc	Necrosis
NPI	Normal Pancreatic Islet
CI	Cellular Infiltration

Abstract

The study was planned to find out a reliable diagnostic techniques for canine diabetes with histopathological effects on different organs. A total number of 53 candidate diabetic dogs from SAQTVH, CVASU and Chittagong metropolitan area were studied. Diabetes in dogs were determined on the basis of clinical finding, hematological profile and serum biochemical profile evaluation. Organs specimens from a dead diabetic and healthy dog were collected for histopathological studies. The prevalence of diabetic dog was 5.76%. The mean blood glucose parameter in diabetic dog was 11.5±0.42 mmol/L. TLC (10.48±0.32 ×103 /µl), ALT (143.38±2.66 IU/L), cholesterol (237.68±0.43 mg/dl) and glycated haemoglobin (9.51±0.31%) values were significantly elevated in diabetic dogs than that of control dog. Glycated haemoglobin (HbA1c %) test was more reliable than portable blood glucose measurement device test, oral glucose tolerance test and hematological profile evaluation test. Histopathologically, the number and size of pancreatic islet were reduced with degeneration, fatty deposition, fibrosis and necrosis. The villus length of intestinal mucosa was shorter (1.1 mm) in diabetic dog than healthy dog (1.5 mm). Cellular infiltration and fatty deposition were observed in liver of diabetic dog. HbA1c test might be useful to clinician for effective diagnosis of canine diabetes and histopathology of different organs might provide knowledge about deleterious effects on organs.

Keywords: Reliable diagnostic techniques, canine diabetes, histopathological effect, organs

Chapter – 1

Introduction

Diabetes mellitus is one of the common metabolic disorders affecting middle-aged to geriatric dogs. It results from absolute or relative deficiency of insulin (Kumar et al., 2014; Guptill et al., 2003). The most common form of diabetes of dog resembles type-1 diabetes in humans (Richard et al., 2014). The prevalence is about 0.4 – 1.21% (Kumar et al., 2014). The classic clinical signs include – persistence fasting hyperglycemia and glycouria (Blood glucose concentration 3.5 to 6.1 mmol/L or 63.1 to 109.9 mg/dl) (Guptill et al., 2003), polyuria, polydipsia, polyphagia, and weight loss (Fledman, 1983), ketonuria and keto-acidosis (ketone levels < 2.8 mmol/L) (Thompson et al., 2001)

The etiology of diabetes in dogs is incompletely understood and may be multifactorial (Hess et al., 2000). An immune-mediated component and environmental factors are mostly involved in the development of diabetes in dogs (Richard et al., 2014). Pancreatitis, obesity, concurrent hormonal disease, hyperadrenocorticism are also considered as risk factor of canine diabetes (Hoenig & Dawe 1992)

The diagnosis is based on the presence of appropriate clinical signs and persistent hyperglycemia and glycosuria, hyperclosterolemia, hypertriglyceridemia and ketonuria and keto-acidosis may develop if the owner fail to recognized the early sings or lax in seeking veterinary care. In serum biochemical profile evaluation, there is a significant increase in the parameters like ALT, cholesterol, triglycerides, fasting blood sugar and glycated hemoglobin values in diabetic dog compared to control dog (Sattar et al., 2007; Andrade et al., 2008; Doxey et al., 1985; Nelson and Reusch 2014). Histoplathologically, the disease is characterized by reduction of number and size of pancreatic islet, decrease the number of beta cells within the islet and beta cell vacuolation and degeneration (Catchpole et al., 2005; Nelson and Reusch, 2014). An extreme form of disease represented by an absolutes deficiency of beta cells and pancreatic islet hypoplasia or aplasia in juvenile dog whereas less severe in adult dog.

Deleterious effects of diabetes in a variety of organs, including vascular, renal, ophthalmic, neurological, digestive and reproductive systems are evaluated (Alberti et al., 2003, Feldman, 1983; Fall et al., 2007). Histologically, intestinal mucosa become atrophic, thicker, reduced villi, stromal infiltration in a diabetic dog (Fall et al., 2007; Jelodar et al., 2007). Fatty deposition, nuclear vacuolation, cellular infiltration and fibrosis are observed in the liver of diabetics (Khan, 1984; Prathaban, 1990).

Several studies are carried out about the diagnosis and effect of diabetes mellitus in dog throughout the world (Fleeman and Nelson, 2001; Kumar et al., 2014; Nelson and Reusch, 2014; Richard et al., 2014). In available literatures a very few studies have been documented on canine diabetes in Bangladesh. Therefore, this study has undertaken for following objectives -

1. To know the current status of canine diabetes
2. To know the reliable diagnostic techniques of canine diabetes
3. To understand the effect of diabetes on different organs

Chapter – 2

Review of literature

2.1 Canine diabetes and its incidence

Ngugi et al. (2012) stated that diabetes mellitus (DM) consists of a group of metabolic diseases that are characterised by a chronic excess of blood glucose, resulting from the defects in insulin secretion, insulin action, or both. It constitutes a major global public health concerns in humans, and as well as in animals.

Souhami et al. (1994) reported, diabetes mellitus is a metabolic disturbance characterized by hyperglycaemia and a relative lack, or complete absence of insulin. It is a disease, which by virtue of its complications may affect all the organs systems in the body.

Kumar et al. (2014) stated that diabetes mellitus is one of the common metabolic disorders affecting middle-aged to geriatric dogs characterized by hyperglycemia, glycosuria and weight loss, resulting from absolute or relative deficiency of insulin. Research conducted on canine diabetes mellitus has direct implication on studying this disease in humans

Fracassi et al. (2004) studied that diabetes mellitus is a common endocrinopathy in dogs, with certain breeds shown to have either an increased or decreased risk of developing the disease. The incidence is unknown, since all published epidemiological studies have been cross-sectional or hospital-based. Their report revealed the prevalence of the disease has varied from 0.3 % to 1.3 %.

Rand et al. (2004) reported that with a prevalence of 0.32% to 0.64%, diabetes mellitus is one of the most common endocrine disorders in middle aged dogs, with a predisposition for female dogs.

Richard et al. (2014) studied that diabetes mellitus is a common disorder in dogs and cats, with a reported hospital prevalence rate of 0.4–1.2%. The most common form of diabetes of dog resembles type-1 diabetes in humans.

Marmor et al. (1982) reported that diabetes in the dog is mostly found in middle aged and older dogs. Its incidence is increasing, possibly due to an increase in obesity in dog. Three different types of diabetes have been identified in dog. Dogs with approximately 40% body fat had normal glucose tolerance and insulin secretion; hyperinsulinemia was seen at higher degrees of obesity and only the group with the highest degree of obesity (approximately 75%) showed glucose intolerance.

Mattheeuws et al. (1984) studied that diabetes mellitus is one of the most common endocrine disorders in dogs, and at present, in man, with polydipsia, polyuria, polyphagia and weight

loss. The prevalence of canine diabetes has been estimated between 0.0005% and 1.5% all over the world.

Feldman and Nelson (1996) found that diabetes mellitus most commonly occurs in middle age to older dogs, but occasionally occurs in young animals. In addition, it occurs more commonly in female dogs than in male.

2.2 Etiology of canine diabetes

Fall et al. (2007) reported that the etiology of diabetes in dogs is incompletely understood and may be multifactorial. Genetic predispositions have been suggested by familial associations, pedigree analysis of Keeshonds, and genomic studies aimed at identification of susceptibility and protective major histocompatibility complex haplotypes.

Jelodar et al. (2007) stated that diabetes mellitus in dog is a chronic metabolic disorder characterized by high blood glucose concentration caused by insulin deficiency, often combined with insulin resistance. It is an endocrine disease in origin, but its major manifestations are those of a metabolic disturbance.

Khan, (1994) reported that diabetes in dog is caused by the impairment of insulin signalling pathways, and the defect usually results from pancreatic β-cell deficiency and/or a deficiency of insulin.

Richard et al. (2014) reported that genetics, an immune-mediated component, obesity concurrent hormonal disease, hyperadrenocorticism and environmental factors are involved in the development of diabetes in dogs.

Kennedy et al. (2006) reported that canine diabetes mellitus is an immune-mediated disease caused by complex interactions between genes and the environment. The disease affects many breeds of dog, including mixed breeds, and susceptibility varies greatly. Researchers suspect that genetic mutations may differ among breeds. Commonly affected breeds include poodles, dachshunds, miniature schnauzers, beagles, puliks and pugs, Cairn terriers and miniature pinschers.

Guptill et al. (2003) reported that certain breeds of dog appear to be predisposed to diabetes. A database containing medical records of over 6,000 diabetic dogs from 24 veterinary schools in North America identified breeds including the Miniature Schnauzer, Bichon Frise, Miniature Poodle, Samoyed and Cairn Terrier as having an increased risk of the disease.

2.3 Clinical signs of canine diabetes

Greco, (2001) found that most diabetic dogs with the classic clinical signs are polyuria and polydipsia. Polydipsia was the most common clinical sign of diabetes mellitus in dogs (93%). Polyuria on the other hand, was observed in only 77% of dogs. Dramatic and rapid weight

loss in an animal with a good or even ravenous appetite will often alert the owner to seek veterinary advice. Weight loss is observed more commonly in dogs (62%). Only 19% of dogs exhibited polyphagia as a clinical sign of diabetes mellitus. In dogs, progressive polyuria, polydipsia and weight loss develops relatively rapidly usually over a period of several weeks.

Foster, (1975) reported that dogs with diabetes mellitus show all the classical symptoms observed in humans. The major complaint at first consultation is usually an increased thirst and increased urination. The owner might also have noticed about weight loss, in spite of a good appetite. Other symptoms may be a dull hair-coat, muscle wasting, tiredness, loss of vision, and infections If the dog is not correctly treated for its disease, it may pass into a ketoacidotic state, which is a severe condition that requires intensive care.

Richard et al. (2014) studied that classic clinical signs of diabetes include polyuria, polydipsia, polyphagia, and weight loss. Clinical signs do not develop until hyperglycemia reaches a concentration that results in glycosuria, typically at blood glucose concentrations of 180–220 mg/dl in dogs and 220–270 mg/dl in cats. A subclinical or prediabetic state as occurs in humans is uncommonly recognized in dogs and cats. The diagnosis of diabetes is based on the presence of appropriate clinical signs and persistent hyperglycemia and glycosuria. Hypercholesterolemia and hypertriglyceridemia are common and ketonuria and ketoacidosis may develop if the owner fails to recognize early signs or is lax in seeking veterinary care.

Briggs et al. (2000) reported, Clinical signs of DM will typically be present when there is persistent hyperglycemia and glucosuria (BG concentration exceed approximately 200 mg/dL in dogs and 250–300 mg/dL in cats). Blood glucose concentrations in these ranges may occur for a variety of reasons, including stress hyperglycemia in cats, corticosteroid administration, the presence of concurrent insulin-resistant disease (hyperadrenocorticism, obesity), or as part of the early stage of developing DM.

Agardh et al. (2005) suggested that insulin deficiency can lead to production of ketone bodies and acidosis with symptoms such as nausea, pain in the stomach, hyperventilation, blurred consciousness and perhaps even unconsciousness and diabetic coma, which is a dangerous acute complication.

2.4 Diagnosis methods of Canine diabetes

Rucinsky, (2010) described different approaches for the diagnosis of diabetes mellitus and assessment depending on the level of hyperglycemia and the presence of clinical signs. For cats and dogs who present with clinical signs suggestive of diabetes mellitus, perform a physical examination and full laboratory evaluation (complete blood count [CBC]),

electrolytes and urine analysis with culture, urine protein:creatinine ratio (UPC), triglycerides, blood pressure (BP), and thyroxine (T4); to confirm the diagnosis as well as to rule out other diseases. Elevated blood glucose can sometimes be identified on blood with the absence of consistent clinical signs. In such cases, if stress hyperglycemia can be ruled out, the patient may be classified as at-risk for developing diabetes mellitus.

Oda et al. (2013) reported, Laboratory evaluation includes a basic minimum database (CBC, chemistry with electrolytes, urine analysis with culture, triglycerides, UPC, BP, and T4 level in cats). Typical findings include hyperglycemia, glucosuria, and stress leukogram, as well as increased cholesterol and triglycerides. Dogs frequently show increased levels of alkaline phosphatase (ALP) and alanine aminotransferase. Cats, however, show more variability in the presence of a stress leukogram and elevated ALP. Elevated liver enzymes in a cat may warrant further evaluation for concurrent liver disease. Pancreatitis is a common comorbidity and may need to be addressed.

Plotnick and Greco, (1995) suggested that the diagnosis of diabetes mellitus in the dog is relatively easy and based on three findings: typical clinical signs (polyuria, polydipsia, weight loss, polyphagia), and persistent fasting hyperglycemia and glycosuria (The combination of persisting fasting hyperglycemia and glycosuria is essential for the diagnosis of diabetes mellitus. A thorough diagnostic work-up is mandatory to exclude or identify concurrent diseases. This work-up should minimally include a thorough physical examination, including oral inspection and ophthalmic examination, complete blood count, serum biochemistry profile, including thyroxine and canine pancreatic lipase immunoreactivity (cPLI) and urinalysis with culture.

Marmor et al. (1982) suggested that diagnosis is based on measurement of glucose in the blood and urine. Healthy dogs do not have detectable amounts of glucose in the urine and have fasting blood glucose concentration between 3.5 and 6.0 mmol/L.2 In general veterinary practice, tests for insulin secretion are not performed. Dogs are sometimes tested for concurrent and potentially diabetogenic disorders, such as hyperadrenocorticism or pancreatitis.

Banting et al. *(*1922) suggested that diagnosis and treatment of DM have largely been based on the measurement of blood glucose concentrations.

2.5 Biochemical changes of blood in canine diabetes

Sattar et al. (2007) suggested that there is a significant increase in the parameters like ALT, cholesterol, triglycerides, fasting blood sugar and glycated hemoglobin values in diabetic dog compared to healthy dog. Glycosylated hemoglobin is formed by an irreversible, non-

enzymatic binding of glucose to hemoglobin. As plasma glucose concentrations increase, hemoglobin glycosylation increases proportionately. Normal glycosylated hemoglobin (mean+/-SD) values are: 2.95 ± 0.15% in dogs. Serum fructosamine is formed by glycosylation of serum protein such as albumin. The concentration of fructosamine in serum is directly related to blood glucose concentration.

Bunn, (1981) reported that the glycosylated hemoglobin (HbA1) assay has gained special importance in the assessment of glycaemic control in diabetic patients It is widely accepted as an objective time averaged index of blood glucose control over the preceding six to eight weeks.

Reusch, (2009) studied found that the glycosylated hemoglobin (GHb) concentration reflects the mean glucose concentration over a period of 10 to 14 weeks in dogs, depending on the life span of red blood cells.

2.6 Histopathological effect of canine diabetes on different organs

A number of reported by various scientist studies that the deleterious effects of diabetes in a variety of organs, including vascular, renal, ophthalmic, neurological, digestive and reproductive systems are evaluated (Alberti et al., 2003; Holstein et al., 2002; De Las Casas et al.,1999). Histologically, intestinal mucosa become atrophic, thicker, reduced villi, stromal infiltration in a diabetic dog Fatty deposition, nuclear vacuolation, cellular infiltration and fibrosis are observed in the liver of diabetics patient in dog

Catchpole et al. (2005) study found that histoplathologically the disease is characterized by a reduction of number and size of pancreatic islets, decrease the number of beta cells within the islet and beta cell vacuolation and degeneration. An extreme form of disease represented by an absolutes deficiency of beta cells and pancreatic islet hypoplasia or aplasia in juvenile dog whereas less severe in adult dog.

Kaneko (1997) study reported, diabetes in dog may cause other organ complications especially eyes, kidneys, heart and blood vessel.

Ling et al. (1977) and Gepts & Toussaint (1967) studies suggested that findings in pancreatic biopsy samples from diabetic dogs have been conflicting. Two fairly large studies of diabetic dogs have shown a reduced number or total absence of islets, together with degeneration, hyalinization or vacuolization of pancreas. Pancreatic biopsy samples from dogs with diabetes mellitus secondary to progestin treatment or diestrus also showed degeneration and vacuolization of the beta cells. Insulitis was not seen in any of these three studies.

Alejandro et al (1988) reported in their study in diabetic dogs characterized by lymphocytic infiltration associated with islets (insulitis) and some dogs displayed extensive exocrine pancreatic damage.

Watson et al. (2007) study showed that chronic pancreatitis is a common finding at autopsies of dogs in general practice. In that particular study 34% of 200 dogs with various diseases were considered as having chronic pancreatitis.

Brenner et al. (2009) studied histopathological investigation in 12 juvenile diabetic Greyhounds dog revealed extensive exocrine and endocrine atrophy of the pancreas.

Chapter - 3

Materials and Methods

3.1 Study area and study population

A total number of 53 dogs from SAQTVH, Chittagong Veterinary and Animal Sciences University (CVASU) and Chittagong Metropolitan area were tested for diabetes by using different diagnostic techniques during the experimental period (January 2017 to January 2018).

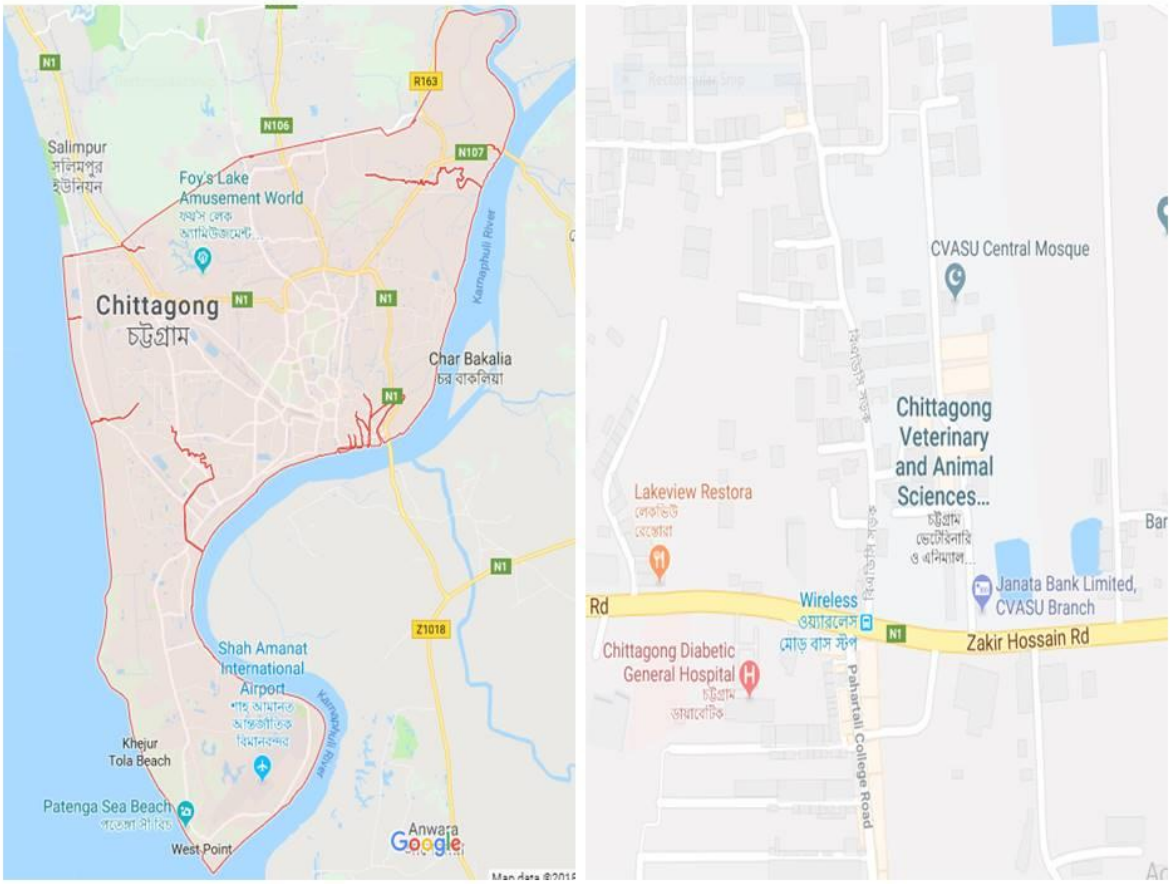

Figure 1: Study area, SQTVH, CVASU, Chittagong metropolitan area

3.2 Experimental design

Candidate diabetic dogs were examined on the basis of clinical finding, hematological and serum biochemical profile evaluations. Dogs were randomly divided into 2 groups; control and diabetic dog. Control dogs were selected from healthy and non-diabetic population. Diabetic doges were 3 – 4 years age and having body weight 5.7±0.28 kg. The age of control dogs were same as diabetic dogs.

3.3 Determination of the disease

The diagnosis of diabetes in dog was based on the measurement of glucose level in the blood and urine. Candidate diabetic dogs were considered on the basis of owner complain, clinical

signs and findings were examined by fasting glucose test and biochemical analysis of blood for further confirmation of the disease. A meeting interview was used for filling in the questionnaire including personal details and clinical characteristics of the studied dogs. All interviews were conducted face to face.

The most reliable clinical parameters were the absence or presence of polyuria, polydipsia, lethargy and weakness. The follow- up of body weight in the medical record was of the utmost importance. Unexpected weight loss and the presence of ketonuria were noticeable in some of the studied dog.

Clinical signs were often suggestive and the diagnosis in dogs was readily confirmed by simple diagnostic tests.

3.3.1Fasting blood glucose level determination by portable blood glucose measurement device

Blood was obtained from the candidate dogs through the standard venipuncture of cephalic vein, saphenous vein and or ear vein to determine their non-fasting blood glucose levels in mmol/L using portable blood glucose measurement device; Glucometer (GLUCO-DOCTOR, China). Food was withdrawn from the dogs overnight. The fasting blood glucose of each dog was then determined at twice in a day.

Blood glucose strips were used to measure blood glucose concentration. A drop of blood was placed on the pad at the end of the strip. After the specified amount of time the pad is wiped and the colour was checked against the chart on the container.

Again a drop of blood was placed on the provided strips, the strip was then inserted into the glucometer and the blood glucose concentration was recorded.

3.3.2 Bio-chemical analysis of blood, urine of candidate dogs for determination of diabetes mellitus

Fasting overnight venous blood samples (about 2 ml each) were collected into vacutainer tubes from studied dogs (both diabetic and healthy). The blood samples were left for a while without anticoagulant to allow blood to clot. Then, serum samples were obtained by centrifugation at room temperature using ROTINA 46 Hettich Centrifuge, Japan at 4000 rpm/10 minutes to be used for biochemical analysis. All the biochemical analysis were done by the procedure described by Kind and King, 1954; Reitman and Frankel, 1957; Varely, 1980 and Meiattini, 1978.

Another 2 ml of blood was collected in tubes containing EDTA to evaluated hematological changes which include: hemoglobin (Hb), packed cell volume, (PCV) and white blood cell count (WBC) as reported by the procedure of Sood, (1996).

Random urine samples were also collected from the same diabetic and control dogs. The samples were then prepared for Benedit test and Rothears test to determine qualitative test of glucose.

3.3.3 Oral glucose tolerance test

The oral glucose tolerance test evaluates clearance from the circulation after glucose loading under defined and controlled conditions. The test has been standardized by the Committee on Statistics of the American Diabetes Association. The studied dogs were fasting for the previous 8-14 hours. A zero time (baseline) blood sample was drawn. The studied dogs were given a glucose solution, which was drunk within 5 minutes. Blood was drawn at intervals for measurement of glucose (blood sugar) according to the study of Belinda, 2004.

3.4 Collection of histopathological specimens

One diabetic dog was died and necropsy was performed by standard methods in order to collect the visceral organs like liver, pancreas, small and large intestine. For control, 2 healthy dogs were sacrificed and the specimens were collected.

All the collected specimens were fixed in bouin's solution and were processed for histopathological studies (Luna et al., 1968).

3.5 Histopathological study of the specimens

Following the customary procedure of paraffin embedding, the tissue blocks were cut in serial cuts, each 5µm thick. For the staining purposes, Mayer's haematoxylin, eosin was used.

3.5.1 Preparation of permanent slide

The preparation of a histological permanent slide includes three main steps

i) Tissue processing: Following steps are involved in the processing of tissue

Fixation/decalcification and washing → Dehydration → Cleaning → infiltration → embedding → sectioning → floating of section in water bath → Attaching of section on glass slide and drying

ii) Staining: After drying, the slide with tissue section is ready for Hematoxilin and Eosin staining

iii) Mounting: Glass slide is protected by cover slip attached to the slide with a mounting medium of appropriate refractive index (e.g., Canada balsam)

3.5.2 Study of histopathological effect on different organs

The qualitative histological analysis of different visceral organs like liver, pancreas, small and large intestine were carried out by an optical microscope (ECLIPSE 400) to which a digital camera had been attached and connected to the control unit and the image projection screen.

Analysis of the stromal structures of different visceral organs, reduction in the number and size of pancreatic islet, the villus length of intestinal mucosa, cellular infiltration and fatty deposition in the liver of diabetic and healthy dog were carried out. Villus length was measured by software along with digital camera.

3.6 Statistical analysis of data

Prevalence of diabetic in dog was calculated with the help of MS Excel 2010, STATA SE 13. The prevalence of diabetes in dog was calculated by the number of cases of diabetic dogs (diagnosed by postmortem and all possible laboratory test) as the numerator and the total number of dead/sick dogs presented as the denominator.

Again the data generated from the haematological and biochemical profile of this study was expressed as mean ± standard deviation of means. Differences between the groups were compared by analysis of variance (ANOVA).

3.7 Photography

All the images related to this study were taken directly from microscope by using the different objectives (4X, 10X).The images were slightly modified for better illustration of the study.

PICTURE GALLARY

Figure 2: Diagnostic tools for diabetes in dog. a. Portable blood glucose measurement device; b. Blood glucose test by PBGMD c. Estimation of ketones bodies by Rothear's test; d. Auto-humanizer for biochemical profile evaluations

PICTURE GALLARY

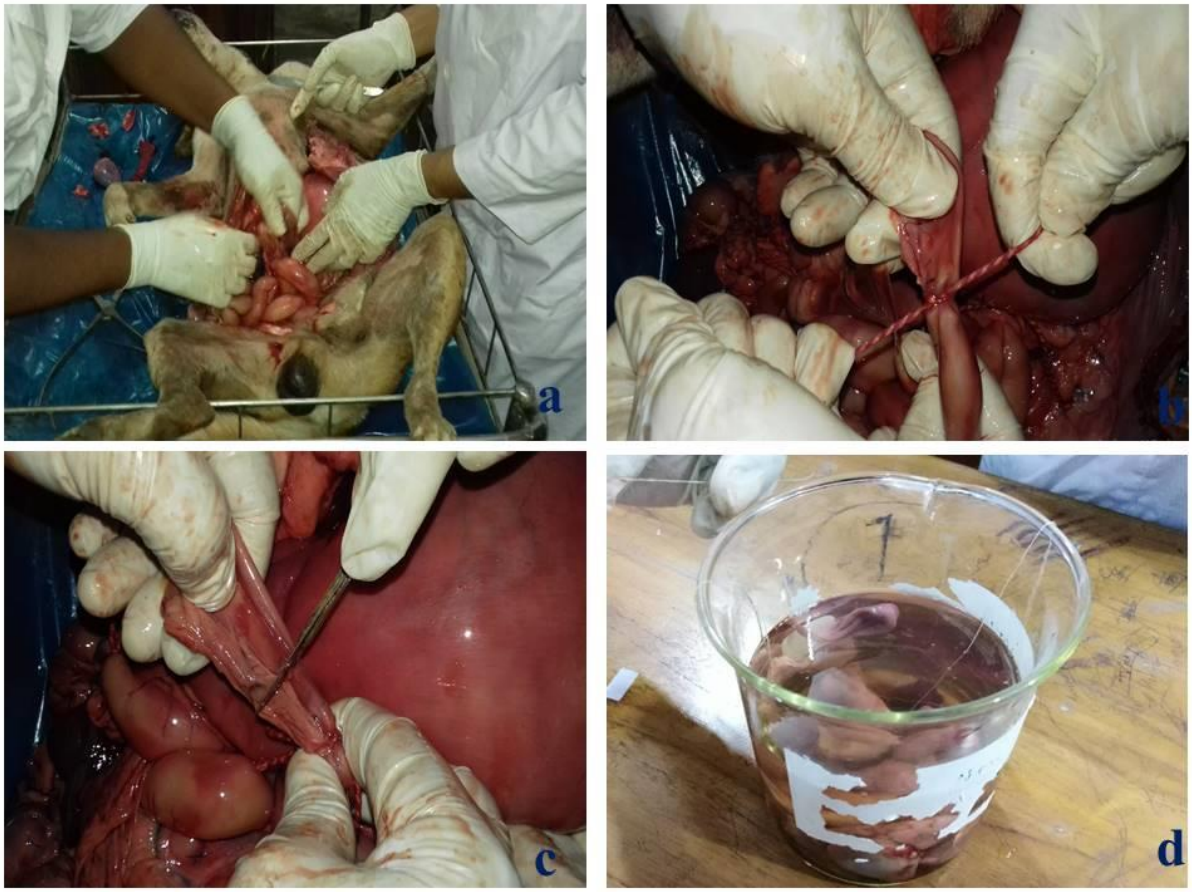

Figure 3: Dissection, collection and preservation of different organs from diabetic and control dog. a. Dissection of dog, b and c. Collection of different organs and d. Preservation of samples

Chapter - 4

Results

4.1 Determination of blood glucose evaluation of candidate dogs by portable blood glucose measurement device

The mean blood glucose parameter in candidate dog was shown in Table 1. The mean value of fasting blood glucose and after meal blood glucose were 11.2±0.08 mmol/L and 11.8 ±0.08 mmol/L in diabetic dogs; respectively. The blood glucose parameter in control dogs were 5.36 ±0.51b mmol/L

Table 1: Blood glucose evaluation in candidate diabetic dog by portable blood glucose measurement device (Data presented are Mean ± SD, n = 53)

Blood glucose parameter in Diabetic dog (n = 3)			**Blood glucose parameter in control dog (n = 50)**	**Reference value**
Morning/before meal	**Afternoon/after meal**	**Mean value**		
11.2 ±0.08 mmol/L	11.8 ±0.08 mmol/L	11.5±0.42[a] mmol/L	5.36 ±0.51[b] mmol/L	3.5 – 6.1

Values are presented as Mean ± SD

Here, SD = Standard Deviation.

4.2 Determination of glucose in blood and urine of candidate dogs by laboratory qualitative testes

The urine samples were prepared for Benedit test and Rothears test to determine qualitative test of glucose in the laboratory and 5 samples were found positive out of 53 samples and shown in Table 2.

Table 2: Laboratory Findings

Blood test	**Urine test**	
Fehling's test (n = 52)	Benedit test (n = 52)	Rothear’s test (n = 52)
Positive sample 5	Positive sample 5	Positive sample 5

Healthy dogs did not have detectable amounts of glucose in the urine and have fasting blood glucose concentration between 3.5 and 6.0 mmol/L (mean value; 5.36 ±0.51 mmol/L).

On the basis of clinical signs, elevated blood glucose parameter and laboratory tests findings the prevalence of canine diabetes was 5.76% which was not statistically significant due to small size of populations.

The most reliable clinical parameters were found as presence of polyuria, polydipsia, lethargy and weakness in the diagnosed diabetic dogs. Unusual body weight loss was noticeable in the diabetic dog.

4.3 Hematological profile evaluation

Hematological profile in normal and diabetic dogs was shown in Table 3 and Figure 4.a. There is no significant difference between healthy and diabetic group with respect to parameters like total leucocytes count (TEC), Platelets count and hemoglobin.

There was a decreased level of total erythrocyte count (7.15±0.13×106 /µl); hemoglobin (10.98±0.17 gm/dl) and increased platelets (3.58±0.05 ×105 /µl) in diabetic dogs compared to healthy dogs.

However there was a significant increase in the total leucocyte count (10.48±0.32×103 /µl) and packed cell volume (38.6±0.37%) in the diabetic group when compared to healthy group.

In control group (non-diabetic healthy dogs), leucocyte count (TEC), total leucocyte count (TLC), Platelet count, hemoglobin and packed cell volume (PCV) were 8.22±0.15×106 /µl; 5.3±0.18×103 /µl; 3.06±0.11×105 /µl; 14.12±1.17 g/dl and 49.73±0.90%; respectively.

Table 3: Hematological profile evaluation

Parameter	Diabetic dog	Control dog	Reference value
TEC ($\times 10^6$ /µl)	7.15±0.13[a]	8.22±0.15[a]	5.5 – 8.5
TLC ($\times 10^3$ /µl)	10.48±0.32[a]	5.3±0.18[b]	6 – 16
Platelet ($\times 10^5$ /µl)	3.58±0.05[a]	3.06±0.11[a]	3 – 3.5
Hemoglobin (g/dl)	10.98±0.17[a]	14.12±1.17[a]	12 – 18
PCV (%)	38.6±0.37[a]	49.73±0.90[b]	37 – 55

Values are presented as Mean ± SD. Means value bearing same superscript are not significantly different at ($P < 0.05$) in the same row when compare to control group.

4.4 Serum biochemical profile evaluation

Serum biochemistry profile in healthy and diabetic dogs was shown in Table 4 and Figure 4.b. There was a significant increase in the parameters like ALT, cholesterol, triglycerides, and glycated hemoglobin values in diabetic group when compared to healthy group. Total protein was decreased significantly compared to the non-diabetic healthy dogs. However the increase in the creatinine value in the diabetic group was not statistically significant.

There was an increased level of ALT (143.38±2.66 IU/L), total cholesterol (237.68±0.43 mg/dl), triglycerides (84.78±0.55 mg/dl), glycated haemoglobin (9.51±0.31%) and decreased total plasma protein (5.98±0.17 gm/dl) concentration in diabetic dogs compared with healthy dogs.

In the control group (non-diabetic healthy dogs), the level of ALT, cholesterol, triglycerides, total plasma protein, glycated haemoglobin were 32.95±0.96 IU/L; 116.12±2.86 gm/dl; 64.41±1.18 gm/dl; 6.53±0.28 gm/dl; 5.2±0.28%; respectively.

Table 4 also showed the levels of creatinine in healthy dogs and diabetes dogs. Creatinine (1.25±0.19 mg/dl) was observed to be higher in diabetic dogs than healthy dogs (0.93±0.02 mg/dl).

Table 4: Serum biochemical profile evaluation

Parameter	Diabetic Dog	Control Dog	Reference value
Creatinine (mg/dl)	1.25±0.19[a]	0.93±0.02[a]	0.8 – 1.00
ALT (IU/L)	143.38±2.66[a]	32.95±0.96[b]	30 - 35
Cholesterol (mg/dl)	237.68±0.43[a]	116.12±2.86[b]	118.85
Triglycerides (mg/dl)	84.78±0.55[a]	64.41±1.18[b]	65 – 70
Total plasma protein (gm/dl)	5.98±0.17[a]	6.53±0.28[b]	5-7.2
Glycated Haemoglobin (HbA1c%)	9.51±0.31[a]	5.2±0.28[b]	6.50 -7.00

Values are presented as Mean ±SD. Means value bearing same superscript are not significantly different at (P <0.05) in the same row when compare to control group.

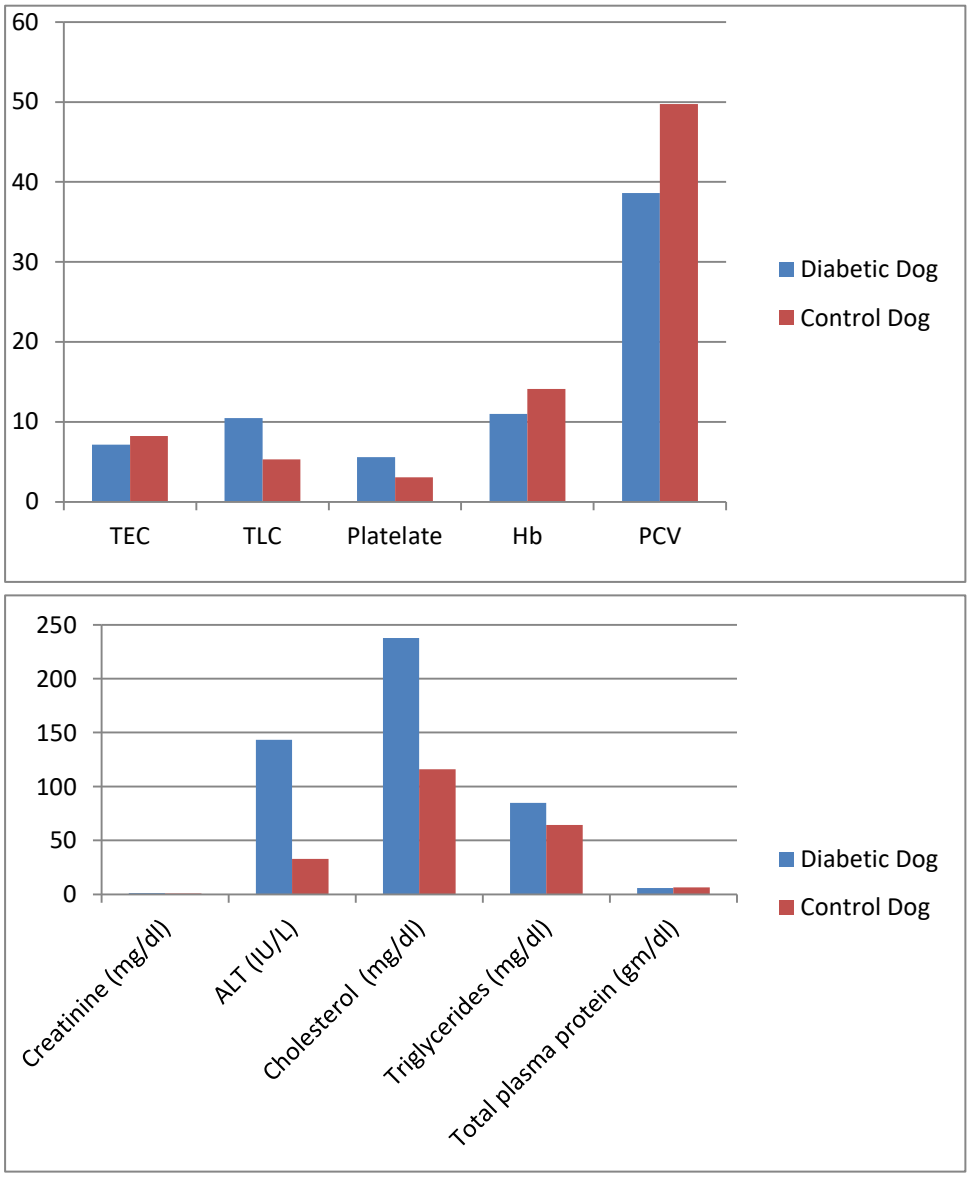

Figure 4: a. Hematological profile of diabetic and control dogs and b. Biochemical profile of diabetic and control dogs

4.5 Oral glucose tolerance test evaluation

Normally, diabetes is detected by measuring blood glucose levels. However, due to wide deviations in the circulating glucose concentrations, a randomized glucose measurement which is a normally used laboratory tests for measuring long-term diabetic control. In addition, diabetic patients have reduced glucose tolerance. Additional burden of glucose is found to weaken the tolerance further. In present study, there was an elevation in blood glucose both fasting and after the meal in take diabetes as compared with normal healthy individuals.

From the result obtained, it was evident that fasting blood glucose levels significantly increase together with corresponding deteriorating oral glucose tolerance in diabetic patients. The intervals and number of samples vary according to the purpose of the test. For simple diabetes screening, the most important sample is the 2-hour sample. The zero and 2-hour samples may be the only ones collected. In a non-diabetic, the level of glucose in the blood goes up (6.9±0.18 mmol/L) immediately after the drink glucose solution. In a diabetic, the glucose in the blood goes up and stays high (12.3±0.14 mmol/L) after drinking the glucose solution. A plasma glucose level of 11.2±0.56 mmol/L or higher at two hours after drinking the glucose solution and at one other point during the two-hour test period confirms the diagnosis of diabetes.

Table 5: Oral glucose tolerance test evaluation of candidate dogs

Blood glucose parameter in diabetic dog			**Reference value**
Zero hour sample	**2 hours sample**	**Mean value**	
10.2 ±0.14 mmol/dl	12.3±0.14 mmol/L	11.25±0.56 mmol/L	3.5 – 6.1
Blood glucose parameter in control dog			
Zero hour sample	**2 hours sample**	**Mean value**	
5.4±0.18 mmol/dl	6.9±0.18 mmol/dl	6.15 ±0.71 mmol/L	

Values are presented as Mean ± SD

4.6 Reliable diagnostic technique

Here, in this study, diabetes in dog was diagnosed based on measurement of glucose in the blood and urine. The most reliable clinical signs were the presence of polyuria, polydipsia, lethargy, weakness and unusual body weight loss of the candidate dog. Those clinical signs were often suggestive and the diagnosis of diabetes in dogs was readily confirmed by simple diagnostic tests like blood glucose evaluation test by portable blood glucose measurement device; hematological profile evaluation test; serum biochemical profile evaluation and oral glucose tolerance evaluation test. Serum biochemical profile evaluation (especially glycated haemoglobin; HbA1c %) test was more reliable than by others testes where oral glucose tolerance evaluation test considered as standard test for diagnosis of diabetes in dog. 3 samples showed higher HbA1c % and they were also found positive in all other's test.

The sensitivity and specificity of different test were calculated and showed in Table 6. Serum biochemistry profile evaluation test (glycated haemoglobin; HbA1c %) showed higher sensitivity (75%) compared to others test and concluded that dog with diabetes were not missed with this test.

Table 6: Sensitivity and specificity of different test for diabetes diagnosis in dog

Test 1	**Diabetes**			**Test 2**	**Diabetes**		
	Positive	**Negative**			**Positive**	**Negative**	
Positive	3	3	PPV=.56	**Positive**	3	1	PPV=.750
Negative	3	44	NPV=.93	**Negative**	1	48	NPV=.97
	SN=50%	SP=93%			SN= 75%	SP= 97%	
Test 3	**Diabetes**			**Test 4**	**Diabetes**		
	Positive	**Negative**			**Positive**	**Negative**	
Positive	3	7	PPV=.3	**Positive**	3	4	PPV=.42
Negative	7	36	NPV=.83	**Negative**	4	42	NPV=.91
	SN=30%	SP=83%		SN=42%		SP=91%	
Test 5	**Diabetes**						
	Positive	**Negative**					
Positive	3	2	PPV= .6				
Negative	2	46	NPV=.95				
	SN= 60%	SP= 95%					

Here, Test 1= oral glucose tolerance evaluation test (6 positive); Test 2 = serum biochemistry profile evaluation test (glycated haemoglobin; HbA1c %; 4 positive); Test 3= blood glucose evaluation test by portable blood glucose measurement device (10 positive), Test 4= hematological profile evaluation test (7 positive) and Test 5 = laboratory findings of blood and urine (5 positive); SN= sensitivity; SP= specificity; PPV= positive predict value and NPV= negative predict value.

4.7 Histopathological effect on different organs

Histopathological studies found the deleterious effects of diabetes in a variety of organs especially pancreas, liver, small intestine and large intestine of the diabetic dog.

In pancreas, there were found reduction in the number and size of pancreatic islet together with degenerative change, hyalinisation or vacuolization was observed (Figure 5).

In addition, the presence of other changes including atypical acinar cell lobules, acini with inspissated secretions, ductular alterations (e.g., epithelial hyperplasia, ductular dilation and inflammation) was also noted. Suppurative inflammation on pancreas of a diabetic dog, there was infiltration of neutrophils within the pancreatic parenchyma or the surrounding peri-pancreatic adipose tissue. Pancreatic necrosis was found. Lymphocytic infiltration principally within the pancreatic parenchyma or the periductular interstitial tissues was noted. Fibrous connective tissue (fibrosis) was incresed within the pancreatic parenchyma (Figure 5).

In liver, Cellular infiltration and fatty deposition in diabetic dog was observed increased in normal compared to the healthy dogs (Figure 6). Nuclear vacuolation and fibrosis was observed in the liver of diabetic dogs (Figure 6).

In kidney, structural changes of glomerulus, destruction of the tubules and fatty deposition was observed (Figure 7).

Histologically, atrophic intestinal mucosa was observed where thicker, reduced villi, stromal infiltration were found (Figure 8 and 9). The length of intestinal villus mucosa was observed shorter (1.1 mm) in diabetic dog compared to normal healthy dog (1.5 mm).

PICTURE GALLARY OF HISTOPATHOLOGICAL FINDINGS

Diabetic dog **Control dog**

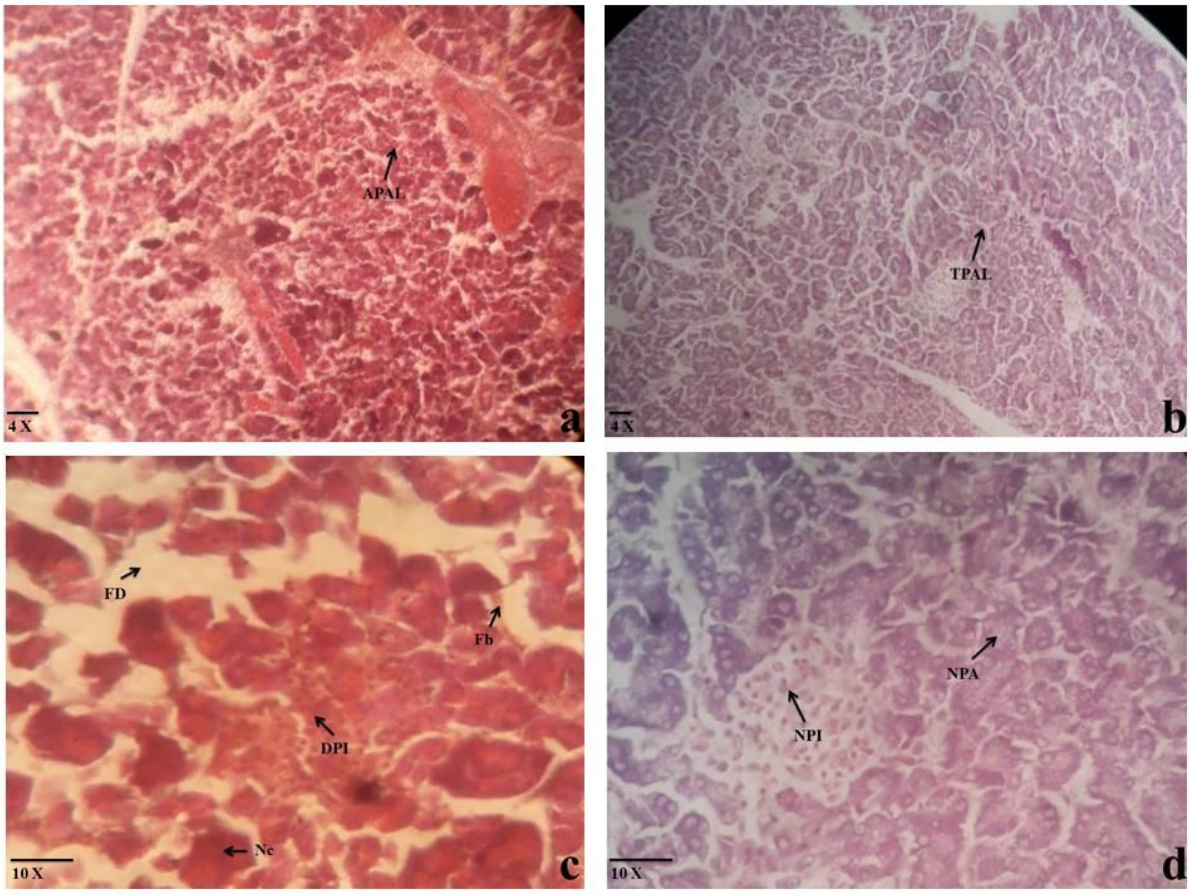

Figure 5: Histopathological effect on pancreas of a diabetic dog (left column) compared with control dog (right column) showing APAL – Atypical pancreatic acinar lobule, TPAL – Typical pancreatic acinar lobule, FD – Fatty deposition, DPI – degeneration of pancreatic Islet, Fb – Fibrosis, Nc – Necrosis, NPI – Normal pancreatic Islet, NPA – Normal pancreatic acini (H&E, 4X and 10X).

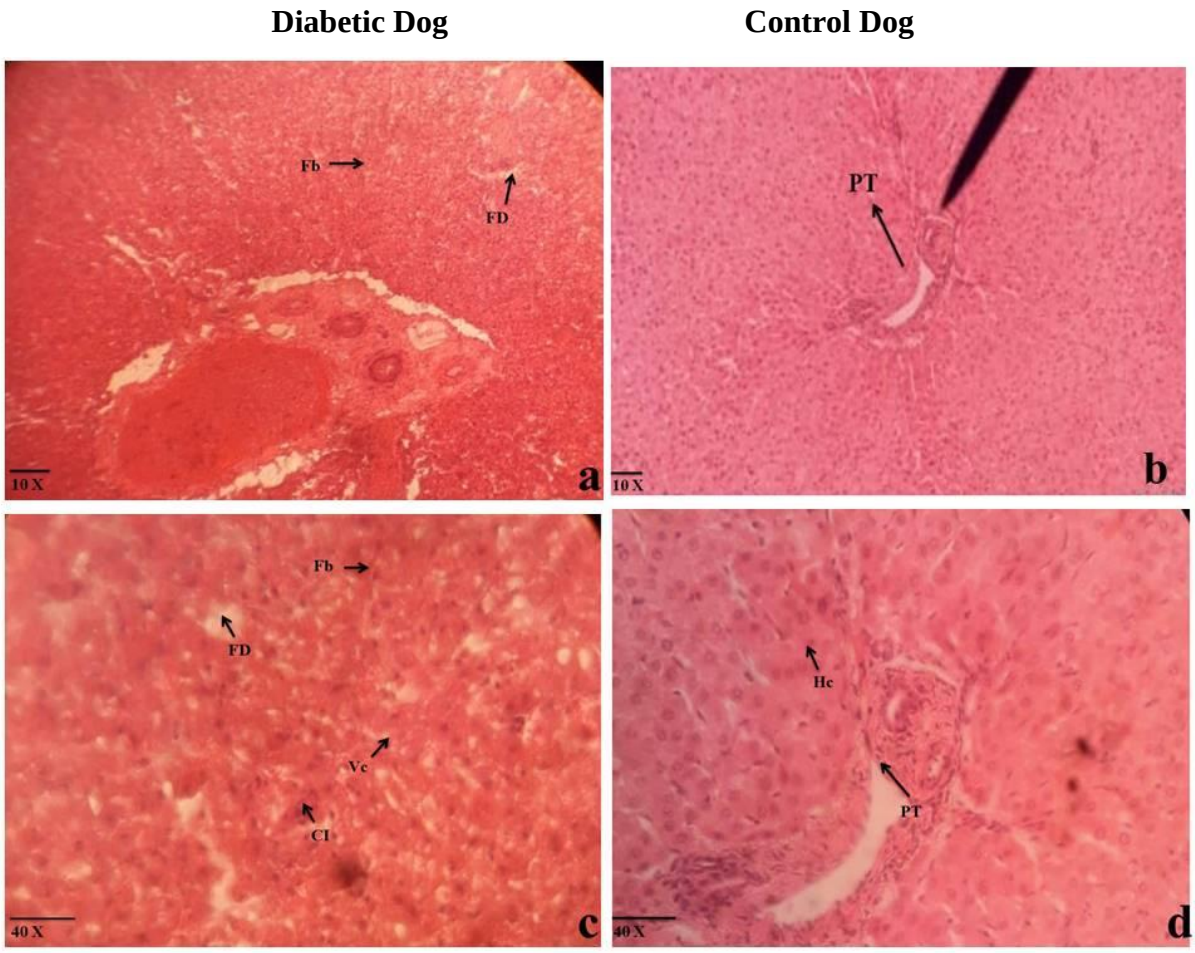

Figure 6: Histopathological effect of liver of a diabetic dog (left column) compared with control dog (right column) showing FD – Fatty deposition, Fb – Fibrosis, CI – Cellular infiltration, Vc – Vaccuolation, Hc – Hepatocyte, PT – Portal tired (arrows) (H&E, 10X and 40X).

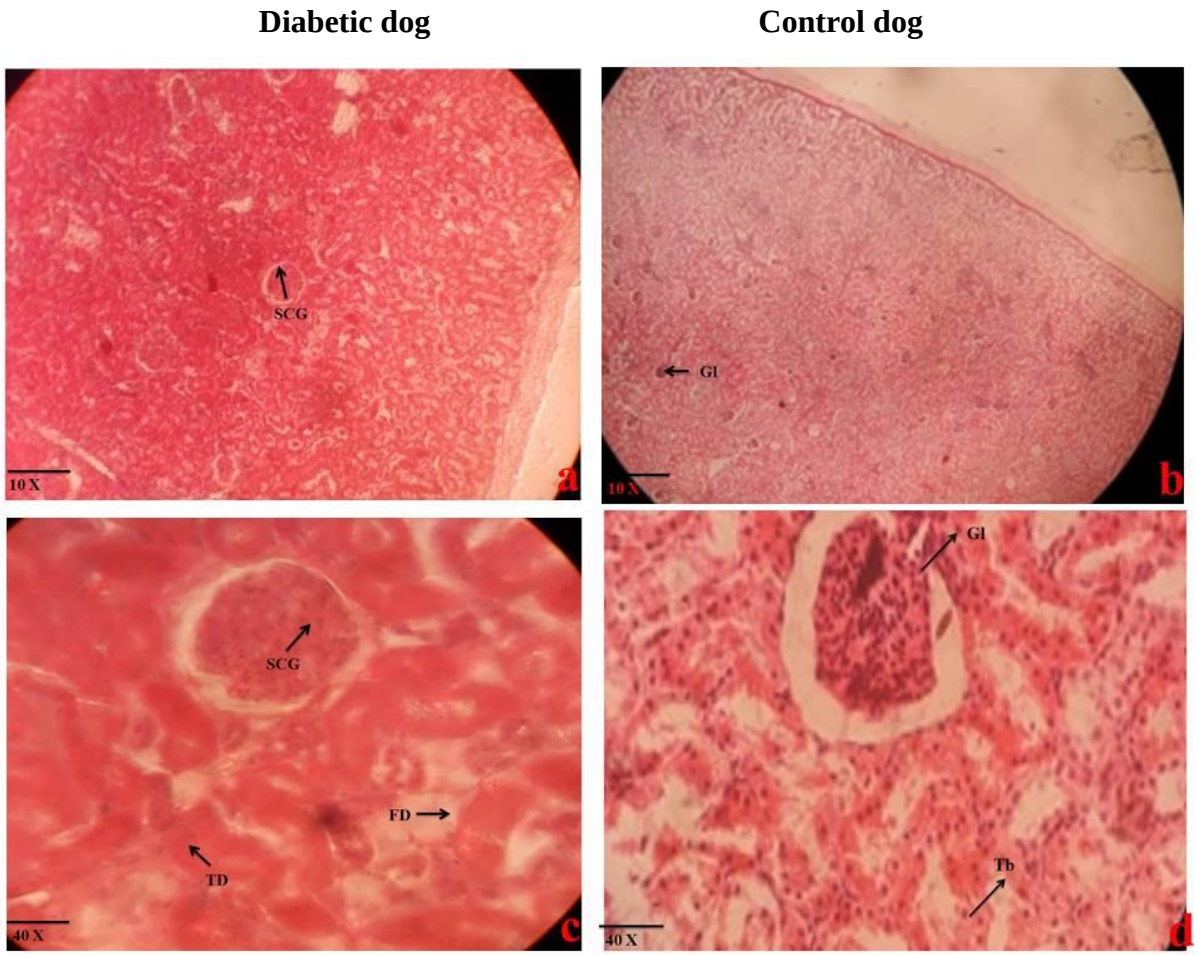

Figure 7: Histopathological effect of kidney of a diabetic dog (left column) compared with control dog (right column) showing SCG – Structural changes of glomerulus, TD – Tubular destruction, FD – Fatty deposition, Gl – glomerulus, Tb – Tubules (H&E, 10X and 40X).

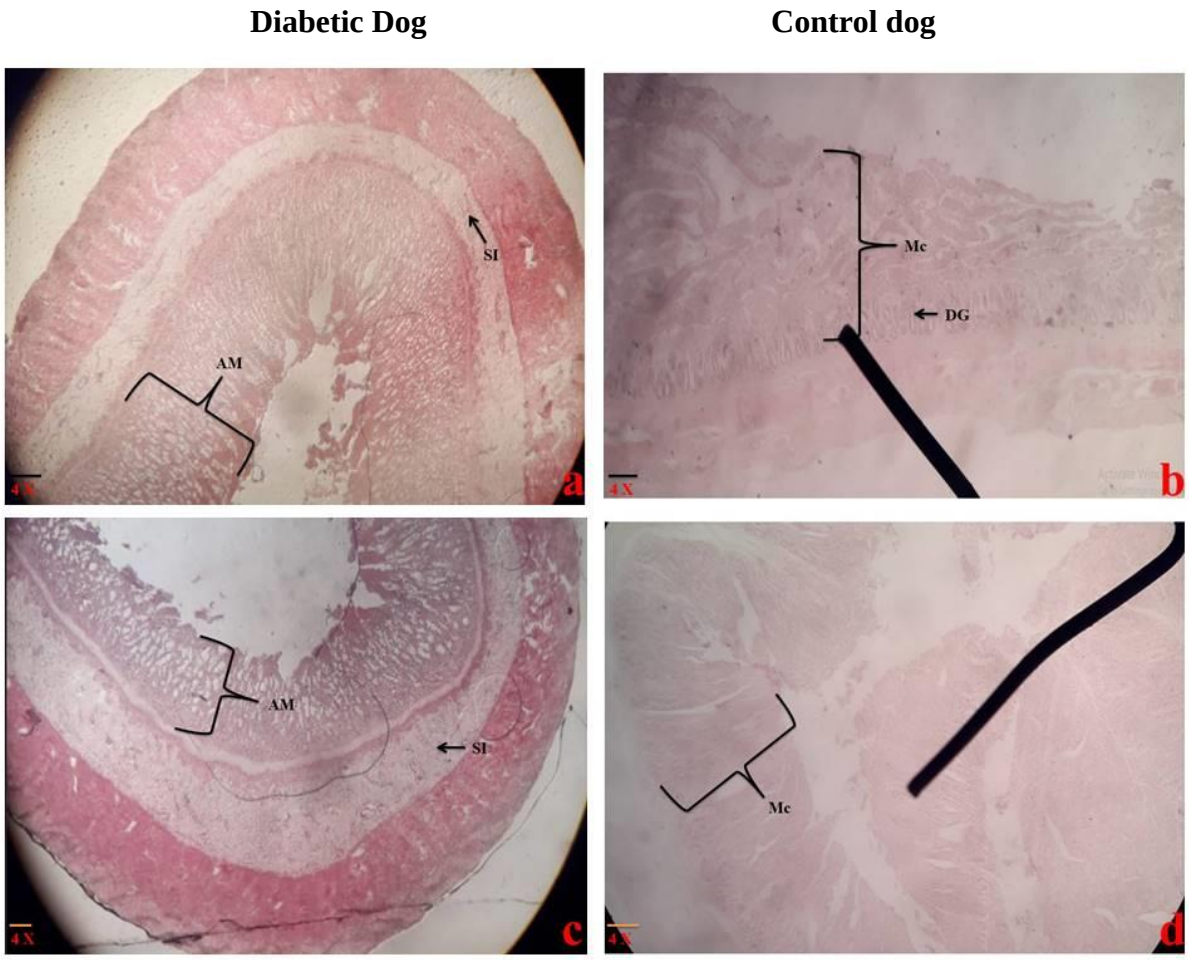

Figure 8: Histopathological effect of small intestine (a,b = duodenum; c,d = jejunum) of a diabetic dog (left column) compared with control dog (right column) showing AM – Atrophied mucosa, SI – Stromal Infiltration (H&E, 4X).

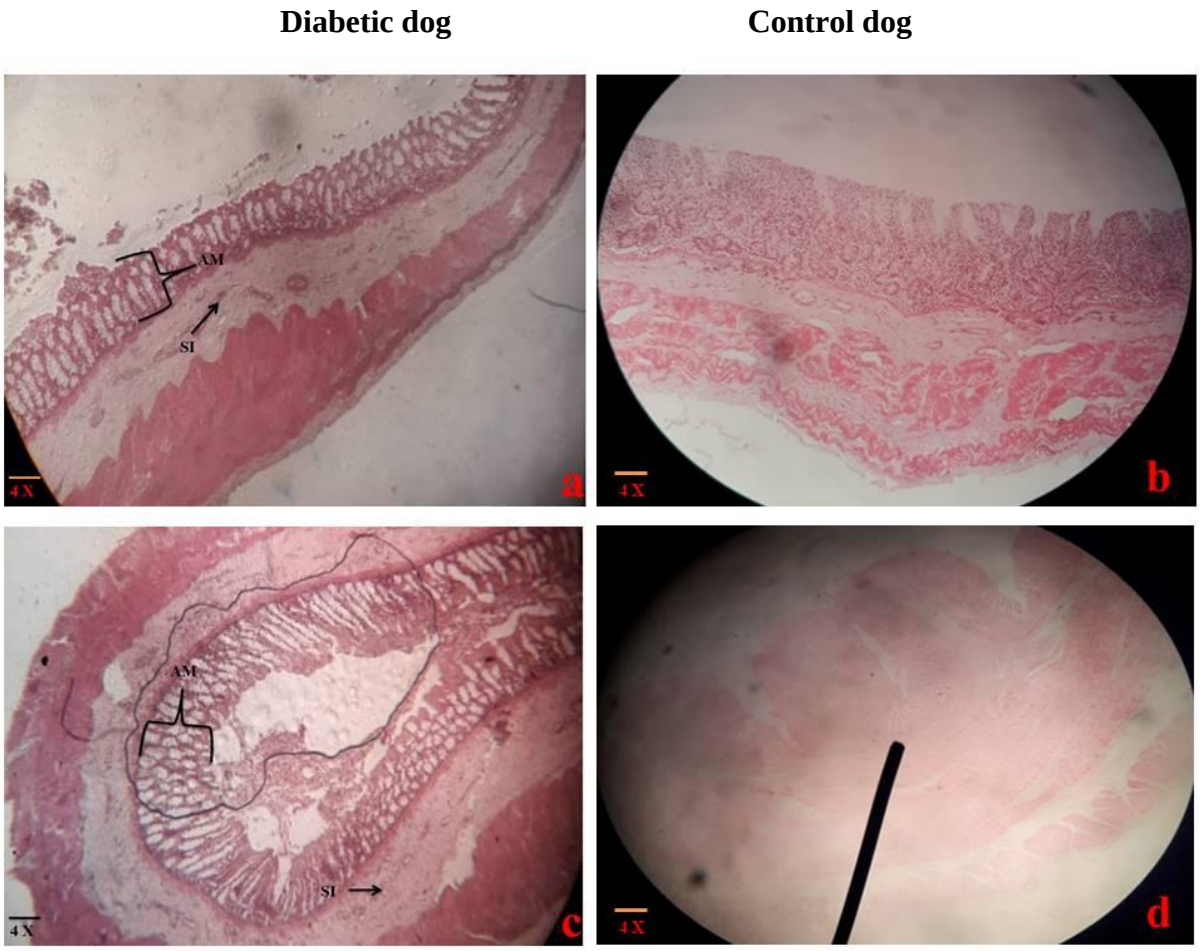

Figure 9: Histopathological effect of large intestine (a = Caecum; b = colon) of a diabetic dog (left column) compared with control dog (right column) showing AM – Atrophied mucosa, SI – Stromal Infiltration (H&E,4X).

Chapter – 5

Discussion

5.1 Clinical signs and prevalence of diabetic

Presence of polyuria, polydipsia, lethargy and weakness in the diagnosed diabetic dogs were most reliable clinical signs compared to healthy dog. Those findings were similar with the findings of Kennedy et al. (2006); Briggs et al. (2000); Richard et al. (2014); Kumar et al. (2014). Unusual body weight loss was noticeable in the diabetic dog in this study and similar findings was reported by Greco, 2001; Bennet, 2002; Van de Maele, 2005. On the basis of clinical signs, elevated blood glucose parameter and laboratory test findings, the prevalence of canine diabetes was 5.76% which was not statistically significant due to small size of population. This findings was higher compared to the study reported by Guptill et al., 2003; Rand et al., 2004; Davison et al., 2005 and Fall et al., 2007; where they found a prevalence of 0.32% to 0.64% in middle aged diabetic dogs. In the study of Fracassi et al. (2004), the prevalence was found 0.3 % to 1.3 %. Again, the prevalence of canine diabetes has been estimated to be anywhere between 0.5% to 1.5% in the study of Mattheeuws et al. 1984

5.2 Blood glucose in diabetic dog

The mean blood glucose parameter in diabetic dog which was tested by Portable Blood Glucose Measurement Device (PBGMD) was 11.5±0.42 mmol/L. This mean value of blood glucose parameter in diabetic dog was similar with the findings of Catchpole et al., 2005; Nelson and Reusch, 2014; where they found mean blood glucose value about 11.87±0.22 mmol/L. The mean blood glucose value observed in this study was also supported by the study of Briggs et al., 2000; Casella et al. 2003; Wiedmeyer and DeClue, 2011; where they found increased glucose concentrations as 12.6 and 13.9 mmol/l; respectively.

The mean value of fasting blood glucose and after the start of meal blood glucose were 11.2 ±0.08 mmol/dl and 11.8 ±0.08 mmol/L; respectively. Those mean values were almost similar with the study of Briggs et al. 2000 and Richard et al. 2014; where they found the mean value of fasting blood glucose 9.7±0.14 mmol/dl and after meal is about 12.15 ±0.29 mmol/L. But in the study of Affenzeller et al. 2010; they found a glucose concentration range about 10.5 mmol/L to 33.3 mmol/L after the meal which made a great variation with the value of present study. Actually, a single glucose measurement (BGM) can be performed with a point-of-care analyzer or a portable blood glucose measurement (PBGM) device. Evaluating a BGM alongside a fructosamine or glycated hemoglobin concentration can help with its interpretation. When interpreting a BGM, it is important to know the timing of the dog's last meal and the insulin dose. Bennet (2002) stated that a single measurement may be

informative when taken at the time of the nadir. However, this is discouraged by Fleeman and Rand (2003), who found a large day-to-day variability in timing of the nadir. Furthermore, a single BGM is highly influenced by stress, excitement, fear or aggressiveness, which may lead to an unreliable measurement (Bennet, 2002; Fleeman and Rand, 2003, Nelson and Couto, 2009).

In the healthy dogs, they did not have detectable amounts of glucose in the urine and have fasting blood glucose concentration usually observed between 3.5 and 6.0 mmol/L (mean value; 5.36 ±0.51 mmol/L). Those values were supported by the references values and also by the values found by Hess et al., 2000; Peterson et al. 2011; Nelson and Reusch, 2014. They found fasting blood glucose concentration between 4.15 to 6.0 mmol/L in healthy dog.

Again, in oral glucose tolerance evaluation test in studied dogs; plasma glucose level of zero hour (10.2 ±0.14 mmol/L) and two hours samples (11.2±0.56 mmol/L) were similar with the findings of Ngugi et al. 2012; where they found 12.6±0.18 mmol/dL at two hours samples collected.

5.3 Hematological profile of diabetic dog

There was a decreased level of total erythrocyte count (7.15±0.13×106 /µl); hemoglobin (10.98±0.17 gm/dl) and increased platelet (3.58±0.05 ×105 /µl) in diabetic dogs compared to healthy dogs. Those findings was supported with the findings of Feldman, 1983; and Deepa et al. 2014; where they found decreased level of total erythrocyte count (7.13±0.37×106 /µl); hemoglobin (11.62±0.44 gm/dl) and increased platelet (3.70±0.39 ×105 /µl). Both findings were almost similar. The decrease in PCV, RBC and haemoglobin concentration might be due to the inflammatory process seen in diabetic condition. The activities of pro-inflammatory cytokines especially, interleukin-6 and tumor necrosis factor alpha have been reported to be contributors of anaemia of inflammatory diseases. Tumor necrosis factor (NF-α) contributes to anaemia of inflammatory diseases by inhibiting erythropoietin secretion and /or response along with direct toxicity on the erythroid precursor cells (Chikazawa and Dunning, 2016).

However there was a significant increase in the total leucocyte count (10.48±0.32×103 /µl) and packed cell volume (38.6±0.37%) in the diabetic group when compared to healthy group. Those findings were more or less similar with the study of Lee et al. 2004; Prathaban, 1990; where they found significant increase in the total leucocyte count ($10.73\pm1.01\times10^3$ /µl) but decreased level of packed cell volume (39.89±1.57%). Leukocytes may be activated through the release of cytokines, such as TNF-α (Shanmugam et al., 2003), transforming growth factor-1 (Korpinen et al., 2001), superoxide (Kedziora-Kornatowska, 1999). Kozlov et al.

(1995) reported that diabetes was accompanied by moderate neutrophilic leukocytosis and prolonged circulation times of neutrophils and monocytes, and a shortened circulation time of lymphocytes, which increases the susceptibility to infection. The raised leukocyte count may also reflect low-grade inflammation.

In control group (healthy dogs), leucocytes count (TEC), total leucocyte count (TLC), Platelets count, hemoglobin and packed cell volume (PCV) were 8.22±0.15×106 /µl; 5.3±0.18×103 /µl; 3.06±0.11×105 /µl; 14.12±1.17 g/dl and 49.73±0.90 %; respectively. Those findings were similar with the references value and almost similar with the previous findings of Feldman, 1983; and Deepa et al. 2014; where leucocyte count (TEC), total leucocyte count (TLC), platelet count, hemoglobin and packed cell volume (PCV) were 8.06 ± 0.46×10^6 /µl; 4.92 ± 0.55×10^3 /µl; 3.09 ± 0.29×10^5 /µl; 12.52 ± 0.67 g/dl and 46.55 ± 2.95 %; respectively.

5.4 Serum biochemical profile of diabetic dog

There was an increased level of ALT (143.38±2.66 IU/L), triglycerides (84.78±0.55 mg/dl), and decreased total plasma protein (5.98±0.17 gm/dl) concentration in diabetic dogs compared with control healthy dogs. The above finding is in agreement with Sattar et al. 2007; and Doxey et al. 1985; where they found increased level of ALT (148.43 ± 35.17 IU/L), triglycerides (85 ± 12.66 mg/dl). Specific elevations in ALT and triglycerides may suggest hepatic fat accumulation as a potential contributing factor for conversion to diabetes in humans. According to the findings of Sattar et al. 2007; if ALT increases and stays high, then there is an association with diabetes risk. In this study, the elevated level of ALT, might be due to hepatic cellular damage leading to leakage of this enzyme into the circulation. The decrease in total plasma protein concentration might be due to degenerative changes in the liver. This decrease could have resulted from dehydration.

There was an increased level of total cholesterol (237.68±0.43 mg/dl) concentration in diabetic dogs compared with control healthy dogs. This finding was supported by the findings of Doxey et al. (1985), Hess et al. (2000) & Nelson and Reusch (2014). According to the findings of Wilkinson, 1960; 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.

Creatinine (1.25±0.19 mg/dl) was observed to be higher in diabetic dogs than healthy dogs (0.93±0.02 mg/dl). This increase in levels of creatinine seen in this study is suggestive of kidney damage which could be due to the cytotoxic effect as reported by Valilou et al., (2007) as well as low kidney perfusion as a result of anaemia.

The increase of glycated haemoglobin (9.51±0.31%) levels in diabetic dogs in the present study when compared to healthy dogs (5.2±0.28%) are also supported by with the findings of Prathaban et al. 1990 and Deepa et al. 2014. In the study of Prathaban et al. 1990; the normal value of glycated hemoglobin (GHb) in healthy dogs was found 6.68 ± 0.37%. The glycated hemoglobin concentration was found to be significantly elevated in the diabetic dogs ranging from 9.6 to 11.4% in this study; which is in agreement with findings of Deepa et al. 2014. They found that diabetic dogs have 9.98% mean HbA1c value. 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.

In the control group (non-diabetic healthy dogs), the level of ALT, cholesterol, triglycerides, Total plasma protein, glycated haemoglobin were 32.95±0.96 IU/L; 116.12±2.86 gm/dl; 64.41±1.18 gm/dl; 6.53±0.28 gm/dl; 5.2±0.28%; respectively. Those findings were supported by the references values and also by Prathaban et al. 1990; Hasegawa et al. 1991; Marca et al. 2000 and Deepa et al. 2014.

5.5 Reliable diagnostic technique

The most reliable clinical signs were the presence of polyuria, polydipsia, lethargy, weakness and unusual body weight loss of the studied dog. Those clinical signs were often suggestive and the diagnosis of diabetes in dogs was readily confirmed by simple diagnostic tests. Single glucose measurement (BGM) was performed by the portable blood glucose measurement (PBGM) device which has given the values of blood glucose concentration. This single BGM is highly influenced by stress, excitement, fear or aggressiveness, which may lead to an unreliable measurement.

The decrease in PCV, RBC and haemoglobin concentration might be due to the inflammatory process seen in diabetic condition. The activities of proinflammatory cytokines especially, interleukin-6 and tumor necrosis factor alpha have been reported to be contributors of anemia of inflammatory diseases. But, anemia may occur due to different causes and different diseases condition. The activities of ALT and higher AST indicates hepatocytes damage which must have resulted from the inflammatory process in hyperglycaemic conditions. The significant correlation observed in healthy dogs and diabetic dogs between glucose and glycated hemoglobin. The 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. Serum biochemistry profile evaluation test (glycated haemoglobin;

HbA1c %) showed higher sensitivity (75%) compare to others test and concluded that dog with diabetes were not missed with this test. 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.

5.6 Histopathological effect on different organs of diabetic dog

There were found reduction in the number and size of pancreatic islet together with degeneration, hyalinisation or vacuolization compared to the pancreas structure of healthy dog. In addition, the presence of other changes including atypical acinar cell nodules, acini with inspissated secretions, ductular alterations was also noted. Those findings were supported by the findings of Catchpole et al. 2005 and Watson et al. 2007. The significant changes in the pancreatic structure have also been recorded by other authors like Ling et al. 1977 and Gepts & Toussaint 1967. In their study, diabetic dogs have shown a reduced number or total absence of islets, together with degeneration, hyalinisation or vacuolisation. According to Alejandro et al. 1988; the diabetic dogs in their study had lymphocytic infiltration associated with islets (insulitis) and that some dogs displayed extensive exocrine pancreatic damage.

In liver, there were found cellular infiltration and fatty deposition in diabetic dog compared to the healthy dogs. There was also found nuclear vacuolation, fibrosis in the liver of diabetic dogs. The above finding was in agreement with Alberti et al. 2003 and Holstein et al. 2002; where they found cellular infiltration and fatty deposition in the liver of diabetic dog.

There were found the structural changes of glomerulus, destruction of the tubules and fatty deposition in kidney of a diabetic dog which were similar with the findings of Alberti et al. 2003

Intestinal mucosa was found atrophied, thicke. Numbers of villi were reduced, stromal infiltration in a diabetic dog was obtained. The villus length of intestinal mucosa was shorter (1.1 mm) in diabetic dog compared to healthy dog (1.5 mm). The above finding was in agreement with Alberti et al. 2003 and Holstein et al. 2002; where they found atrophied, thicker, reduced villi in intestine of diabetic dogs.

Chapter - 6

Conclusion

Now- a-days, dog become popular pet animal in Bangladesh. The owners are more conscious regarding the health status of their dog than the owners of the others pet animals. Among metabolic diseases, diabetes mellitus are common in dog. The deleterious effects of the disease are related with behavior of the dogs and normal function of vital organ system. Therefore, determinations of diabetic status of the dog are important for proper treatment, care and management. In this study, the reliable diagnostic technique (glycated haemoglobin; HbA1c %) might be useful to clinician for effective diagnosis of canine diabetes and histopathology of different organs might provide knowledge about deleterious effects on organs due to diabetes mellitus.

Chapter – 7

Recommendations and future perspectives

Finding of the result would be useful to the clinician for effective diagnosis of canine diabetes. The study would be continuing further in a wide range of population with long time for the better understanding of the disease.

References

Alberti S, Spadella CT, Francischone TRCG, Assis GF, Cestari TM, Taveira LAA. 2003. Exfoliative cytology of the oral mucosa in type II diabetic patients: morphology and cytomorphometry. Journal of Oral Pathology and Medicine. 32(9): 538-543.

Agardh CD, Cilio, CM, Lethagen A, Lynch K, Leslie RD, Palmer M, Harris RA, Robertson JA, Lernmark A. 2005. Clinical evidence for the safety of GAD65 immunomodulation in adult-onset autoimmune diabetes. Journal of Diabetes Complications. 19(4): 238-46.

Alejandro R, Feldman EC, Shienvold FL, Mintz DH. 1988. Advances in canine diabetes mellitus research: etiopathology and results of islet transplantation. Journal of American Veterinary Medical Association. 193(9), 1050-5.

Andrade-Cetto A, Martinez-Zurita E, Soto-Constantino A, Revilla-Monsalve C, Wiedenfeld H. 2008. Journal of Ethnopharmacol. 116(2), 358-62.

Banting FG, Best CH, Collip JB, Fletcher AA. 1922. Pancreatic Extracts in the Treatment of Diabetes Mellitus. The Canadian Medicine Association Journal. 12: 141-146.

Bunn HF. 1981. Evaluation of glycosylated haemoglobin in diabetic patients. Diabetes. 30: 613-617

Brenner K, Harkin KR, Andrews GA, Kennedy G. 2009. Juvenile pancreatic atrophy in Greyhounds: 12 cases (1995-2000). Journal of Veterinary Internal Medicine. 23(1): 67-71.

Catchpole B, Ristic JM, Fleeman LM, Davison LJ. 2005. Canine diabetes mellitus: can old dogs teach us new tricks? Journal of Veterinary. 48(10): 1948–1956.

Belinda R. 2004. Gale Encyclopaedia of Alternative Medicine. 2603-2605.

Briggs CE, Nelson RW, Feldman EC. 2000. Reliability of history and physical examination findings for assessing control of glycemia in dogs with diabetes mellitus: 53 cases (1995-1998). Journal of American Veterinary Medical Association. 217(1):48–53.

Bennet N. 2002. Monitoring techniques for diabetes mellitus in the dog and the cat. Clinical Techniques in Small Animal Practice. 17: 65-69.

Casella M, Wess G, Hässig M. 2003. Home monitoring of blood glucose concentration by owners of diabetic dogs. Journal of Small Animal Practice. 44: 298-305.

Chikazawa S, Dunning MD. 2016. A review of anaemia of inflammatory disease in dogs and cats. Journal of Small Animal Practice. 57: 348-353.

Davison LJ, Ristic JM, Herrtage ME, Ramsey IK, Catchpole B. 2003. Anti-insulin antibodies in dogs with naturally occurring diabetes mellitus. Journal of Veterinary ImmunolImmunopathol. 91(1): 53–60.

Deepa PM, Dimri U, Jhambhi R, Ramees TP, Vijaykumar H, Gopinath D, Mahendran K, Mondal DB. 2014. Secondary subclinical diabetes mellitus in dogs infected with Ehrlichiacanis. International Journal of Advance Research. 2 (1): 858-863.

Doxey JE, Milne EM, Mackenzie P 1985. Canine diabetes mellitus: a retrospective survey. Journal of Small Animal Practices. 26:555-561.

Feldman EC. 1983. Diseases of the endocrine pancreas. In: Text Book of Veterinary Internal Medicine. Diseases of the dog and cat. Edt. Ettinger, S. J. Edn. 2nd., W.B. Saunders Company, Philadelphia, London.

Fall T, Hamlin HH, Hedhammar A, Ka¨mpe O, von Euler H. 2007. Diabetes mellitus in a population of 180,000 insured dogs: incidence, survival, and breed distribution. Journal of Veterinary Internal Medicine. 21:1209–1216. (doi:10.1111/j.1939-1676.2007.tb01940.x)

Foster SJ. 1975. Diabetes mellitus--a study of the disease in the dog and cat in Kent. Journal of Small Animal Practices. 16(5): 295-315.

Fracassi F, Pietra M, Boari A. 2004. Breed distribution of canine diabetes mellitus in Italy. Veterinary Research Community. 28(Suppl):339–342.

Feldman EC, Nelson RW. 1996. Diabetes mellitus. In: Feldman EC, Nelson RW (eds) Canine and feline endocrinology and reproduction, 2nd ed, WB Saunders, Philadelphia, pp. 330-391.

Guptill L, Glickman L, Glickman N. 2003. Time trends and risk factors for diabetes mellitus in dogs: analysis of veterinary medical database records (1970–1999). Journal of Veterinary 16(5): 240–247.

Gepts W, Toussaint D. 1967. Spontaneous diabetes in dogs and cats. A pathological study. Diabetologia. 3(2): 249-65.

Greco DS. 2001. Diagnosis of diabetes mellitus in dogs and cats In: Vet Clin North Am (EN Behrend and RJ Kemppainen, eds). Vol 31,845-853, Philadelphia WB Saunders.

Hess RS, Kass PH, Ward CR. 2000. Breed distribution of dogs with diabetes mellitus admitted to a tertiary care facility. Journal of American Veterinary Medical Association. 216(9): 1414–1417.

Hoenig M, Dawe DL. 1992. A qualitative assay for b cell antibodies. Preliminary results in dogs with diabetes mellitus. Veterinary Immunology and Immunopathology 32: 195–203.(doi:10.1016/0165-2427(92)90046-S)

Haberer B, Reusch CE. 1998. Glycated hemoglobin in various pathological conditions: Investigations based on a new, fully automated method. Journal of Small Animal Practices. 39(11): 510-517.

Hasegawa S, Sako T, Takemura N, Koyama H Motoyoshi S. 1991. Glycated hemoglobin fractions in normal and diabetic dogs measured by high performance liquid chromatography. Journal of Veterinary Medical Science. 53(1): 65-68.

Jelodar G, Razmi N, Gholampour V. 2007. Arginase alteration in the reproductive system of alloxan-diabetic dogs. The Journal of Reproduction and Development. 53: 317- 321.

Kahn CR. 1994. Banting Lecture: Insulin action, diabetogenes and the cause of type II diabetes. Diabetes. 43: 1066-1084.

Korpinen E, Groop PH, Fagerudd JA, Teppo AM, Akerblom HK, Vaarala O. 2001. Increased secretion of TGF-beta1 by peripheral blood mononuclear cells from patients with type 1 diabetes mellitus with diabetic nephropathy. Diabet Medicine. 18:121-125.

Kedziora-Kornatowska KZ. 1999. Production of superoxide and nitric oxide by granulocytes in non-insulin-dependent diabetic patients with and without diabetic nephropathy. IUBMB Life. 48:359-62.

Kozlov IA, Novitski VV, Baìkov AN. 1995. Kinetics of blood leukocytes in mice with alloxan diabetes. Biull Eksp Biological Medicine. 120:33-35.

Kind PRN, King EJ. 1954. Journal of clinical pathology 7: 322 , cited by Wooton L.D. P. and Freeman H . (1982) . Microanalysis in medical biochemistry, 6th ed., Churchill Livingstone.

Kennedy LJ, Davison LJ, Barnes A, Short AD, Fretwell N, Jones CA. 2006. Identification of susceptibility and protective major histocompatibility complex haplotypes in canine diabetes mellitus. Journal of Small Animal Practices. 68(6): 467–476.

Kumar P, Kumari RR, Jumar M, Kumar S, Chakravbarti A. 2014. Current practices and research updates on diabetes mellitus in canine. Journal of Veterinary World. 7(11): 952–959.

Kaneko JJ. 1997. Carbohydrate metabolism and its disease. In: Kaneko J, Harvey JW, Bruss ML (eds) Clinical biochemistry of domestic animal, 5th ed, Academic Press, California, pp. 71.

Ling GV, Lowenstine LJ, Pulley LT, Kaneko JJ. 1977. Diabetes mellitus in dogs: a review of initial evaluation, immediate and long-term management, and outcome. Journal of American Veterinary Medical Association. 170(5), 521-30.

Luna LG. 1968. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. 3rd edn., Mc Graw Hill Book Co., New York.

Lee FT, Cao Z, Long DM, Panagiotopoulos S, Jerums G, Cooper ME. 2004. Interactions between angiotensin II and NF-kappaBdependent pathways in modulating macrophage infiltration in experimental diabetic nephropathy. Journal of Animal Society of Nephrology. 15: 2139-51.

Marmor M, Willeberg P, Glickman LT, Priester WA, Cypess RH, Hurvitz AI. 1982. Epizootiologic pattern of diabetes mellitus in dogs. Am. J. Vet. Res. 43: 465 - 470.

Meiattini F. 1978. The 4-hydroxy benzoate/4-amino phenazone chromogenic system. Clinical Chemistry. 24: 2161-2165.

Marca MC, Loste A. 2000. Glycosylated Hemoglobin Assay of Canine Blood Sample, Journal of Small Animal Practices. 41: 189-192.

Mattheeuws D, Rottiers R, Kaneko JJ, Vermeulen A. 1984. Diabetes mellitus in dogs: relationship of obesity to glucose tolerance and insulin response. Animal Journal of Veterinary Research. 45:98–103

Marmor M, Willeberg P, Glickman LT, Priester WA, Cypess RH, Hurvitz AI. 1982. Epizootiologic patterns of diabetes mellitus in dogs. Am J Vet Res. 43(3): 465- 470.

Nelson RW, Reusch CE (2014). Animal models of disease: classification and etiology of diabetes in dogs and cats. Journal of Endocrinology. 222(3): 1–9.

Nelson RW. 2000. Oral medications for treating diabetes mellitus in dogs and cats. Journal of Small Animal Practices. 41(11): 486-490.

Nelson RW, Couto CG. 2009. Disorders of the endocrine pancreas. In: Small Animal Internal Medicine. Fourth Edition, Mosby/Elsevier, St. Louis, 767-783.

Ngugi MP, Njagi JM, Kibiti CM, Ngeranwa JJN, Njagi ENM. 2012. Diagnosis of Diabetes Mellitus. International Journal of Diabetes Research. 1(2): 24-27 DOI: 10.5923/j.diabetes.20120102.01

Oda H, Mori A, Lee P 2013. Characterization of the use of liraglutide for glycemic control in healthy and Type 1 diabetes mellitus suffering dogs. Research on Veterinary Science. 95(2):381–8.

Prathaban S. 1990. Study on clinical and clinic pathological aspects of canine diabetes mellitus. Ph. D. thesis, Tamil Nadu Veterinary and Animal Sciences University, Madras, India.

Plotnick AN, Greco DS. 1995. Home management of cats and dogs with diabetes mellitus. Common questions asked by veterinarian and client. Veterinary Clinics of North America: Small Animal Practice. 25: 753-759.

Peterson ME, Nesbitt GH, Schaer M. 2011. Diagnosis and management of concurrent diabetes mellitus and hyperadrenocorticism in thirty dogs. Journal of American Veterinary Medical Association. 178: 66 - 69.

Reitman S, Frankel S. 1957. A colorimetric method for determination of serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase. American Journal of Clinical Pathlogy. 2: 28:56.

Richard W, Nelson R, Reusch CE. 2014. Classification and etiology of diabetes in dogs and cats. Journal of Endocrinology. 222:3, T1–T9. http://joe.endocrinology-journals.org DOI: 10.1530/JOE-14-0202

Rand JS, Fleeman LM, Farrow HA, Appleton DJ, Lederer R. 2004. Canine and feline diabetes mellitus: nature or nurture? The Journal of Nutrition 134 (supplement), 2072S-2080S.

Rucinsky R, Cook A, Haley S. 2010. AAHA diabetes management guidelines for dogs and cats. Journal of American Animal Hospital Association. 46(3):215–24.

Reusch CE. 2009. Diabetic Monitoring. In: Bonagura J.D. and Twedt D.C. (Editors). Kirk's Current Veterinary Therapy XIV.W.B. Saunders, Philadelphia, p. 209-213.

Sattar N, MCconnachie A, Ford I, Gaw A, Cleland SJ, Nita G, Orouhi MC, Farlane P, Shepherd J, Cobbe S Packard C. 2007. Serial Metabolic Measurements and Conversion to Type -2 Diabetes in the West of Scotland Coronary Prevention Study. Specific Elevations in Alanine Aminotransferase and Triglycerides Suggest Hepatic Fat Accumulation as a Potential Contributing Factor. Diabetes. 56:984-991

Souhami RJ, Moxham M. 1994. Diabetes Mellitus and disorders of lipid and intermediary metabolism. Churchill Livingston, London.

Sood R. 1996. Hematology for students and practitioners.4th ed., Jaypee brothers, New Delhi, India.

Shanmugam N, Reddy MA, Guha M, Natarajan R. 2003. High glucose-induced expression of proinflammatory cytokine and chemokine genes in monocytic cells. Diabetes. 52:1256-64.

Thompson MD, Taylor SM, Adams VJ, Waldner CL, Feldman EC. 2002. Comparison of glucose concentrations in blood samples obtained with a marginal ear vein nick technique versus from a peripheral vein in healthy cats and cats with diabetes mellitus. Journal of American Veterinary Medical Association. 221(3):389–392.

Varely H. 1980. Practical clinical biochemistry.4th ed., William Heinman medical book, London. Pp:802

Van de Maele I, Rogier N, Daminet S. 2005. Retrospective study of owners' perception on home monitoring of blood glucose in diabetic dogs and cats. The Canadian Veterinary Journal. 46: 718-723.

Valilou MR, Sohrabi HR, Mohammadnejad D, Soleimani RJ. 2007. Histopathological and ultra-structural lesions study of kidney of Alloxan induced diabetes mellitus in German shepherd dogs. Journal of Animal and Veterinary Advances. 6 (8): 1012-1016.

Williams TL, Peak KJ, Brodbelt D, Elliott J, Syme HM. 2012. Survival and the development of azotemia after treatment of hyperthyroid cats. Journal of Veterinary Internal Medicine. 24(4): 863- 869.

Watson PJ, Roulois AJ, Scase T, Johnston PE, Thompson H, Herrtage ME. 2007. Prevalence and breed distribution of chronic pancreatitis at post-mortem examination in first-opinion dogs. Journal of Small Animal Practices. 48(11): 609-618.

Wilkinson JS. 1960. Spontaneous Diabetes Mellitus. Vet. Rec. 72(28): 548-555.

Wiedmeyer CE, DeClue AE. 2011. Glucose monitoring in diabetic dogs and cats: adapting new technology for home and hospital care. Clinics in Laboratory Medicine. 31: 41-50.

Appendix

Questionnaire for data collection of a diabetic suspected dog

Owner's name: ……. Date: …….

Address: ……

Owner's mobile number: ……

Information about the dog:

a) Breed: …… b) Age of dog: …….

c) Sex: …… d) Body weight: ……..

General health:

a) Dog health is: Excellent/ Very good/ Good/ Fair/ Poor

b) Symptoms:

1. Fatigue in the past 2 weeks: …….
2. Shortness of breat in the past 2 weeks: ……
3. Increase thirst? ……..….. No/ Yes/ Don't know
4. Decrease appetite? ……..……. No/ Yes/ Don't know
5. Nausea or vomiting? …….. No/ Yes/ Don't know
6. Abdominal pain? …… No/ Yes/ Don't know
7. Frequent urination at night? (3 or more times)….. No/ Yes/ Don't know

Diabetes history: (If tested before)

a) Year/ age of diabetes diagnosis: ……..

b) Types of diabetes: Type I/ Type II/ Pre-diabetes/ Gestational/ Don't know

c) Any weight changes (up or down)? Yes/ No

If yes, please explain: …….....

d) Take care or management of diabetes? Yes/ No

If yes, how long ago? …….

Checking blood glucose:

a) Blood sugar range: …….

b) How often? i. Once a day ii. 2 or more/day iii. 1 or more/week iv. Occasionally

c) When: i. before meal ii. 2 hours after meal iii. Night time

Meal plan:

a) Meal plan for a dog: Yes/ No.
   If yes, please describe: ……
b) About how aften do you use this meal plan for your dog? Never/ seldom/ sometimes/ usually/ always
c) Do you have any dietary restrictions for your dog? Salt/ fat/ fluid/ none/ other
d) Sample of dog meals for a typical day:
   Time: ……. Breakfast: ……..
   Time: ……. Lunch: …….
   Time: ……. Dinner: ……..
   Time: …….. Snack: ……..

Physical activity:

a) Exercise regularly? Yes/ No
b) Type of exercise for your dog: ……
c) How often? ……..

Other medical concerns:

Eyeproblems/ kidney problems/ dental problem/ high blood pressure/ high cholesterol/ sexual problems/ depression/ others ……..
…….

Medication:

a) Any diabetes medication for dog: Yes/ No
   Diabetes pills/ insulin injections/ combination of pills and injections
b) Others medication? Yes/ No
   List other medications: ……..
   ……..
   …….

Biography

Sonnet Poddar was born 25th December 1991 at Pirojpur Sadar, Pirojpur, Bangladesh. His father's name is Sharat Chandra Poddar and mother's name is Laxmi Rani Poddar. He has passed his Secondary School Certificate (SSC) examination from Kiamuddin High School, Pirojpur Sadar, Pirojpur in 2006 and Higher Secondary Certificate (HSC) examination from Govt. Suhrawardy College, Pirojpur Sadar, Pirojpur in 2008. He has obtained his Doctor of Veterinary Medicine (DVM) Degree in 2014 from Chittagong Veterinary and Animal Sciences University, Khulshi, Chittagong, Bangladesh. During undergraduate period he has received clinical training on veterinary medicine from Tamilnadu Veterinary and Animal Sciences University, India and also from Khonkaen University, Thailand. Now, he is a candidate for the degree of MS in Anatomy under the department of Anatomy and Histology, Faculty of Veterinary Medicine, CVASU. He has published seven scientific articles, one national and six in international journal. He has immense interest to work in molecular biology.