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**Faculty of Veterinary Medicine**  
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**Evaluation of Hepatocyte-Derived microRNA-122(miR-122) For  
Diagnosis of Selected Hepatobiliary Diseases of Dogs.**

**Thesis presented by**

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**2019**

**Supervision sheet**

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## **Abstract**

Hepatobiliary diseases are commonly encountered in canine medicine and can be classified into parenchymal, vascular, biliary or neoplastic diseases. Diagnosis of liver diseases is considered a challenge for a veterinarian and requires a lot of diagnostic workup as specific clinical signs become overt only when hepatocellular damage became massive so new sensitive, non invasive hepatic diagnostic biomarkers are needed. In this study 64 dogs were used, 15 of them were used as a normal control group, 10 of them were used for induction of steroid hepatopathy and 39 of them suffered from hepatobiliary diseases. Diagnostic workup were recorded including clinical examination, hematology, serum biochemical constituents, abdominal ultrasonography, US guided fine-needle liver biopsy for cytological and histopathological investigations and serum hepatocyte derived miRNA-122 analyzed by real-time quantitative polymerase chain reaction. In conclusion, serum hepatocyte derived miRNA-122 is of diagnostic value and highly stable, non invasive, easily measurable blood indicator for detection of hepatocellular injury in dogs in addition to miRNA-122 highly significant elevated in acute hepatitis, chronic hepatitis, canine multicentric lymphoma involving liver, slightly elevated in steroid induced hepatopathy and moderate elevated in biliary diseases.

**Key Words:** Canine, Hepatobiliary diseases, Hemato-biochemical analysis, miRNA-122, Ultrasonography, Liver biopsy.

## *DEDICATION*

*I dedicate this work to my parents and sisters for all the support they  
lovely offered during my post-graduate studies.*

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## List of Abbreviations

|             |   |
|-------------|---|
| <b>ACD</b>  | Anemia of chronic disease               |
| <b>ADH</b>  | Antidiuretic hormone                    |
| <b>AH</b>   | Acute hepatitis                         |
| <b>ALP</b>  | Alkaline phosphatase                    |
| <b>ALT</b>  | Alanine aminotransferase                |
| <b>AST</b>  | Aspartate aminotransferase              |
| <b>BUN</b>  | Blood urea nitrogen                     |
| <b>CBC</b>  | Complete blood count                    |
| <b>CH</b>   | Chronic hepatitis                       |
| <b>CHC</b>  | Chronic hepatitis C                     |
| <b>CL</b>   | Canine lymphoma                         |
| <b>CPSS</b> | Congenital portosystemic shunts         |
| <b>CT</b>   | Cycle threshold                         |
| <b>DIC</b>  | Disseminated intravascular coagulopathy |
| <b>HBV</b>  | Hepatitis B virus                       |
| <b>HCA</b>  | Hepatocellular adenomas                 |

|                     |                                  |
|---------------------|----------------------------------|
| <b>HCC</b>          | Hepatocellular carcinomas        |
| <b>HCV</b>          | Hepatitis C virus                |
| <b>HD-miRNA-122</b> | Hepatocyte-derived microRNA-122  |
| <b>HDmiRs</b>       | Hepatocyte-derived microRNAs     |
| <b>HVPG</b>         | Hepatic venous pressure gradient |
| <b>L</b>            | Lymphoma                         |
| <b>miRNAs</b>       | microRNAs                        |
| <b>MU</b>           | Mucoceles                        |
| <b>PD</b>           | Polydipsia                       |
| <b>PH</b>           | Primary hepatitis                |
| <b>Ph</b>           | Portal hypertension              |
| <b>PU</b>           | Polyurea                         |
| <b>RBCs</b>         | Red blood cells                  |
| <b>RH</b>           | Reactive hepatopathy             |
| <b>SIH</b>          | Steroid induced hepatopathy      |
| <b>US</b>           | Ultrasound                       |
| <b>WHO</b>          | World health organization        |

## *Chapter (1)*

### **Introduction**

# Chapter (1)

## Introduction

Liver diseases are frequently encountered problems in small animal practice (Cullen, 2009) and usually associated with non specific clinical signs including depression, lethargy, anorexia, vomiting and diarrhea (Richter, 2003). Specific clinical signs only recognized in an advanced stage when hepatocellular damage became severe due to large reserve capacity of the liver and treatment at this stage are less effective (Verzija *et al*, 2014).

Dogs with liver disease remain for long period subclinical in addition, these subclinical animals often show normal enzymatic levels; so that they are difficult to be diagnosed by current screening methods (Fieten *et al*, 2012). New hepatic diagnostic biomarkers are needed to overcome this lack in sensitivity of current screening methods (Li *et al*, 2012).

Recently, hepatocyte-derived microRNAs (miRNAs) became stable and sensitive diagnostic blood biomarkers for liver illnesses in human and animal models (Laterza *et al*, 2009; Wang *et al*, 2009; Zhang *et al*, 2010 and Van der Meer *et al*, 2013).

MicroRNAs are cluster of small noncoding RNAs that play vital roles in hepatic functions and imperative regulators of post-transcriptional gene expression (Krol *et al*, 2010). MicroRNA-122 is specific indicator for hepatocellular injury as it represents 72% of all miRNAs population in liver (Dirksen *et al*, 2016).

Liver specific miRNA-122 has an essential function in the maintenance of cellular homeostasis in hepatocytes and important roles in tumor suppression, reducing hepatic inflammation and lipid metabolism (Hsu *et al*, 2012 and Tsai *et al*, 2012). miRNAs recently were shown to be detectable and highly stable in the circulation, so can be used as blood biomarkers (Gilad, 2008 and Mitchell, 2008).

**Therefore the present thesis was carried out to**

- Evaluate clinical findings, hematology, serum liver biochemical constituents, abdominal ultrasonography, cytology, histopathology of liver tissue obtained by US guided needle biopsy and serum miRNA-122 in apparently healthy dogs, acute hepatitis, chronic hepatitis, canine multicentric lymphoma, portal hypertension, cholecystitis and cholelithiasis.
- Induction of steroid hepatopathy then evaluate clinical findings, hematology, serum liver biochemical constituents, abdominal ultrasonography, cytology, histopathology of liver tissue obtained by US guided needle biopsy and serum miRNA-122 in it.