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Evaluation of Hepatocyte-derived MiRNA-122, and Insulin-like Growth Factor 1 for the Diagnosis of Liver Diseases in Dogs

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Abstract

THIS study aimed to evaluate the value of hepatocyte-derived miRNA-122 and insulin-like growth factor 1(IGF-1) in the diagnosis of liver affections in dogs. The study was carried out on 122 dogs of different breeds, sex, and ages, divided into 4 groups, acute hepatitis (AH), chronic hepatitis (CH), cholangitis (CHL), and control (C) groups. All dogs were subjected to clinical, hematological, biochemical, and ultrasonographic examinations. AH diseased dogs showed various clinical signs including jaundice and fever, and a significant decrease in IGF-1. Dogs with CH showed weight loss, dullness, ascites, and a significant ($P \leq 0.001$) decrease in RBCs count and Hb content. Dogs with AH and CH showed a significant ($P \leq 0.001$) elevation of ALT, AST, IL6, MDA, CRP, and a significant ($P \leq 0.001$) decrease of albumin, SOD, and GSH. Serum miRNA-122 analysis revealed a significant increase in the AH, CH, and CHL groups. Ultrasonographic findings revealed a diffused decrease in echogenicity of liver parenchyma in the AH group, but a diffused increase in echogenicity of liver parenchyma in the CH group. The current study concluded that hepatocyte-derived miRNA-122 is a promising molecular biomarker for the diagnosis of liver affections in dogs. In addition, IGF-1 is a useful biomarker for the diagnosis of AH disorders.

Keywords: Dogs, Liver, Hepatocyte-derived miRNA-122, IGF 1, Ultrasonography.

Introduction

The liver achieves several important functions such as the metabolism of fat, glycogen, lipids, and proteins, storage of vitamins, trace minerals, fat, and immune regulation. The liver is highly susceptible to damage due to its function as a filter for portal blood, endogenous metabolites, and exposure to foreign substances [1]. The most prevalent liver disease in dogs is hepatitis, which can be either in acute or chronic form. There is a high incidence of chronic hepatitis, which may or may not be associated with cirrhosis [2]. Liver illness in dogs stays asymptomatic for a very long time, and the identification of hepatic disorders is challenging since many clinical signs only occur in association with extensive hepatocellular damage [3]. Furthermore, these subclinical animals frequently exhibit normal enzymatic levels making it challenging for present screening methods to diagnose them [3]**.** Therefore, a comprehensive diagnosis process involving a clinical examination, an ultrasonographic examination, and laboratory testing is required [4].

The most practical diagnostic imaging method for identifying hepatobiliary diseases is abdominal ultrasonography which can identify changes within the liver parenchyma and compare liver tissue to other soft tissues by displaying the changes in tissue structure as differences in echogenicity [5]. Several biomarkers have been recently introduced to diagnose hepatic affections in dogs. One of these markers is insulin-like growth factor 1 (IGF-1), a small polypeptide hormone having 70 amino acids, which is crucial for both prenatal and postnatal developments. The liver produces the majority of serum IGF-1 upon growth hormone stimulation, and it has a strong affinity for IGF-1 binding proteins [6]. MicroRNAs, a class of noncoding RNAs, are highly stable in blood and play a significant role in distinct cell processes. Circulating microRNAs have been studied in humans as noninvasive indicators of liver inflammation, fibrosis, and cancer [7]. MiR-122, the most prevalent microRNA in the liver, is used as a marker of hepatic diseases in humans [8]**.** Circulating miR-122 may be helpful as a sensitive biomarker for early identification of liver injury in dogs [9].

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Therefore, the current study was designed to investigate the diagnostic value of Hepatocyte-derived miRNA 122 and IGF1 for the diagnosis of various liver diseases in dogs combined with clinical, hematobiochemical and ultrasonographic evaluations.

Material and Methods

Ethical approval

All examinations were done after the approval of the Ethics Committee of the Faculty of Veterinary Medicine, Benha University, Egypt, with the approval number (BUFVTM 11-01-23).

Animals

This study was carried out from August 2022 to December 2023 on 122 dogs of different breeds, sex, and ages admitted to Pet Animals Veterinary Clinic, Faculty of Veterinary Medicine, Benha University, and to private pet animals' clinics in Cairo governorate, Egypt.

A preliminary screening was performed for each dog to evaluate the liver status. It included obtaining a case history, measuring body temperature, pulse, and respiratory rates, and examining the color of the mucous membrane. Dogs having clinical signs of ascites, anorexia, dullness, vomiting, diarrhea, melena, and jaundice were exposed to clinical, hematobiochemical, and ultrasonographic examinations for dog classification into different liver affections [10]. Accordingly, dogs were allocated into acute hepatitis (AH, n=19), chronic hepatitis (CH, n= 84), and cholangitis (CHL, n=9) groups in addition to normal healthy dogs (C; n=10) which were kept as a control group.

Collection of samples

After the owner's consent, blood samples were collected from the cephalic vein in a sterile K3EDTA-containing tube. Besides 5 ml of blood was collected in a sterile plain tube without an anticoagulant for serum separation and analysis of biochemical parameters.

Hematological analysis

An automated hematology analyzer (Model No.93-91098-00-GF) was used to determine total erythrocytic count (RBCs), hemoglobin concentration (Hb), packed cell volume (PCV), total leukocytic count (WBCs), and differential leukocytic counts.

Biochemical analysis

The total protein (TP), albumin, total Bilirubin, direct bilirubin, blood urea Nitrogen (BUN), creatinine, alanine amino transferees (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), triglycerides, cholesterol were analyzed using specific kits according to manufacturer instructions (Spectrum Diagnostic Kits, Egypt). Malondialdehyde (MDA), Superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) were analyzed according to manufacturer instructions (Bio-diagnostic Company, Giza, Egypt).

The concentration of IL-6 was determined using the Canine IL-6 Duoset ELISA (catalog number DY1609; R&D Systems, Inc.). The analysis included plate preparation, and the assay procedure was performed according to the manufacturer's ELISA protocol. Finally, the microtiter plate was read using a microtiter reader (SLT Spectra, Tecan) at 450 nm.

Serum CRP concentration was measured by ELISA kit according to the method described by [11].

For IGF-1 assay, serum samples were frozen at -20 C till the time of examination (Vetmed Labor, Ludwigsburg, Germany). IGF-1 concentrations were assayed using a radioimmunoassay (DSL-5600 Active IGF-1 coated-tube IRMA-Kit; Diagnostic Systems Laboratories, Webster, TX, USA) as per manufacturer instructions. The assay is mainly used for human serum but can be applied to dogs due to the amino acid sequences of dogs and human IGF-1 are similar [12].

Hepatocyte-derived miRNA-122:

Serum samples stored at −20 till miRNAs-122 were measured [3]. Briefly, RNA from samples was extracted using RNeasy Mini Kit (Catalogue No. 74104, QIAGEN, Germany) according to manufacturer guidelines. Quantitative polymerase chain reaction (PCR) for the determination of miRNA-122 was done using Quantitect SYBR Green PCR Kit (QIAGEN, Germany, Cat. No. 204141), oligonucleotide primers and probes used in SYBR Green Real-time PCR are shown in Table (1) [3] and cycling conditions for SYBR Green Real-time PCR are shown in Table (2). Amplification curves and cycle threshold (CT) values were measured by the Stratagene MX3005P software, and the CT of each sample was compared with that of the control group to estimate the variation of gene expression on the RNA of different samples according to the "ΔΔCt" method stated by [13] using the following ratio: ($2^{-\Delta\Delta Ct}$)

Whereas ΔΔCt = ΔCt reference – ΔCt target

ΔCt target = Ct control – Ct treatment and ΔCt reference = Ct control- Ct treatment

Ultrasonographic examination

Abdominal ultrasonography was performed for the evaluation of liver parenchymal tissue, portal,

and hepatic blood vessels, the biliary system, and the gallbladder using a CHISON ECO ultrasound machine with 3.5-5.5 MHz convex probe, the gain was 100, and depth 6.9 cm, and the guidelines for classification of liver affections were made according to the method mentioned by [14].

Statistical analysis

The obtained results were expressed as mean ± SE and analyzed using one-way ANOVA to define the significant variance between different groups followed by pairwise comparison using the Tukey post hoc test (SPSS Statistics for Windows, version 28.0. Armonk, NY: IBM Corp). Pearson correlation was computed to obtain the direction and strength of the relationship between the different biochemical parameters.

Results

Clinical findings

Dogs with AH showed signs of fever and dullness. Moreover, there was yellowish discoloration of the conjunctival and oral mucous membranes, and inner aspect of the ear. Dogs with CH showed signs of weight loss, pale mucous membrane, ascites, diarrhea, and dehydration. Dogs with CHL showed nonspecific clinical signs of depression, dullness, and diarrhea.

There was a significant ($p< 0.001$) increase in body temperature, and pulse and respiration rates in the acute hepatitis group only, but there was an unremarkable change in body temperature, pulse rate, and respiration in the CH, and CHL groups (Table 3).

Hematological findings

There was a significant ($p<0.001$) decrease in RBCs count and PCV% in the AH and CH. Hb content significantly ($p<0.001$) decreased, but platelet count increased in the CH. There was a significant increase ($p <0.001$) in WBCs count in all groups (Table 4).

Biochemical findings

The ALT level showed a highly significant increase ($p<0.001$) in the AH, and CH groups, and the level of AST level showed a highly significant increase ($p<0.001$) in the AH group. The ALP level showed a highly significant increase ($p<0.001$) in the AH and CH groups (Table 5).

The creatinine level exhibited a significant increase ($p<0.001$) in the CH group. The blood urea nitrogen (BUN) level showed a high significant increase in CH, AH, and CHL groups (Table 5).

The level of total protein showed a highly significant ($p<0.001$) decrease in the CHL, CH, and AH groups. The level of albumin showed a highly significant ($p< 0.001$) decrease in the CH group (Table 5).

The level of total bilirubin showed a highly significant ($p< 0.001$) increase in AH, CH and CHL groups. The level of direct bilirubin showed a highly significant ($p< 0.001$) increase in the CHL group (Table 5).

The MDA level showed a highly significant ($p< 0.001$) increase in the CH, and AH groups. The level of SOD and GSH-Px showed a highly significant decrease ($p< 0.001$) in CH and AH groups (Table 5).

The level of IL6 and CRP showed a significant ($p< 0.001$) increase in AH and CH groups (Table 5).

The level of IGF-1 showed a highly significant ($p< 0.001$) decrease in the AH and CHL groups (Table 5).

There was a significant positive correlation between liver enzymes and MDA, IL6, CRP, ALP, and BUN and a negative correlation between liver enzymes and SOD, GSH, and IGF1 in AH and CH groups. The level of IGF-1 negatively correlated with levels of ALT, AST, IL6, and CRP in AH and CH groups (Table 6).

Hepatocyte-derived miRNA-122

The serum hepatocyte-derived miRNA-122 values showed 10.05-fold increase in AH group, 7.16-fold increase in CH group, and 2.25-fold increase in CHL group (Table 7).

Ultrasonographic examination

The dogs affected with AH showed a diffused decrease in echogenicity of the liver tissue when compared with the renal cortex (Figure 1). There was a diffused increase in echogenicity of the liver parenchyma with thickening and dilatation of hepatic veins in dogs affected with chronic hepatitis (Fig. 2).

The dogs affected with CHL showed poorly defined echogenic material consistent with biliary sludge within the gall bladder (Figure 3).

Discussion

In the current study the diagnostic value of hepatocyte-derived miRNA-122, and IGF-1 hormone were investigated in dogs with different hepatic affections to overcome the lack of reliability of the ordinary screening methods. Hepatocyte-derived miRNA-122 is detectable in the serum as a molecular biomarker of liver functional status [15]. Since circulating IGF-1 hormone is mainly produced in the liver, it has been proposed that it is correlated with liver disorders [16].

The clinical examination of the dogs with liver diseases showed signs of weight loss that could be due to inappetence and increased tissue breakdown. The observed pale mucous membrane could be attributed to anemia [17]. Moreover, dogs with acute hepatitis suffered from jaundice which may be owing to impairment of the liver's ability to remove bilirubin [2]. Dogs with chronic hepatitis showed ascites which could be related to decreased albumin assembly or intrahepatic portal hypertension [18]. These clinical findings were parallel to [17] and [1].

The possible causes of the reduction in RBC count and Hb content may be low portal blood flow, decreased bone marrow response, decreased RBCs survival time, decreased food intake due to inappetence or off food, and decreased availability of micronutrients from the liver for the formation of hemoglobin [19]. The observed significant reduction in PCV% in the present study might be due to the dehydration that occurred in the dogs suffered from various liver diseases [4]. Increased WBCs count in this study could be a consequence of hepatocellular injury, absorption of intestinal bacterial toxins in addition to stress [20], these results were similar with [2], and [19].

The biochemical results in the current study revealed a significant increase in ALT level in AH group, and positive correlation between liver enzymes and MDA, IL6, and CRP, and a negative correlation between liver enzymes and SOD, GSH, and IGF-1. The increased level of ALT could be owing to the majority of ALT is found in the cytoplasm of hepatocytes, and it is released into the serum in cases of hepatic dysfunction or increased hepatocyte membrane permeability [20]. Similar findings were observed by [21], and [2].

The increased level of creatinine and BUN in the CH group might be attributed to renal malfunction which pointed out as a common complication in cases of hepatic affection due to the liver's capacity to detoxify toxic substances being compromised [19]. These findings coincided with those reported by [19].

The observed hypoproteinemia is the most common finding in chronic hepatitis as the liver is the main site for protein metabolism, also most plasma proteins are produced and broken down by the liver, making them a sensitive sign of impaired liver function. The low serum albumin level because of hepatic illness indicated diffuse and chronic hepatic disorders [19]. These findings were similar with [2] and [22].

Hyperbilirubinemia in acute hepatitis in this study could be due to a disturbance of the balance between the rate of production, metabolism, and excretion of bilirubin [23], and these findings were similar to [2] and [4].

The elevated level of ALP in this study was believed to be a sign of either intrahepatic or extrahepatic biliary blockage [22], these findings were similar to [2], who reported that a significant increase in ALP level in acute hepatitis in dogs, and [24], who found that high level of ALP in dogs with hepatic disease.

In the current study, there was a significant increase in MDA level, and a significant decrease in SOD and GSH activities. The level of MDA positively correlated with levels of liver enzymes. Although, the levels of SOD and GSH negatively correlated with liver enzymes. The decreased activity of SOD was due to depletion by reactive oxygen species during the detoxifying process. However, the decrease in the level of GSH could be owing to increased oxidant challenge [25]. Increased level of MDA in our study could be due to long-term oxidative stress caused by the oxidation of cytotoxic free fatty acids. Furthermore, increased lipid peroxidation produces byproducts like MDA, which have been demonstrated to further promote the production of cytokines [26], similar findings have been reported by [27].

The increased level of serum IL6 in the AH and CH groups in the current study were in accordance with [28] as IL6 is an acute phase inflammatory mediator motivated by activated monocytes, macrophages, and Kupffer cells, fibroblasts, and endothelial cells [29].

Serum CRP levels showed a significant increase in the AH and CH groups and positively correlated with ALT, AST, and IL6 levels. It is important to know that high serum CRP concentrations are not specific for liver disorders and have been reported in a wide variety of diseases in dogs [30], similar findings were reported by [31].

Hepatic affections are known to alter the serum concentration of insulin-like growth factor-1 (IGF-1) in humans, but such effect was rarely investigated in dogs [16]. In the present study, the level of IGF-1 showed a significant decrease in the AH group. Moreover, there was a negative correlation between the level of IGF-1 and AST, IL6, and CRP in the current study. These findings might be owing to a decreased number of functional hepatocytes in the injured liver or a decreased number of growth hormone receptors on the cell surface of damaged hepatocytes [12].

The dogs affected with AH showed a diffuse hypoechogenicity of the liver tissue perhaps due to passive congestion of the liver, but in the CH group diffuse hyperechogenicity of liver tissue may be due

to fibrosis of the liver [32], and these findings were similar to [2]. Ultrasound findings in dogs with cholangitis showed poorly defined echogenic material consistent with biliary sludge within the gall bladder with thickening in the wall of the gall bladder, and these findings were similar to [33].

Hepatocyte-derived miRNA-122 is known to play a significant role in the preservation of cellular homeostasis in hepatocytes. It may change in association with hepatic inflammation, tumor, and lipid metabolism. So, hepatocyte-derived miRNA-122 is considered a promising biomarker for hepatocellular damage as miRNA-122 accounts for 72% of all miRNAs located in liver [9]. In the result presented there was an elevation in the level of hepatocyte-derived miRNA-122 in the dogs with HT, AH, and CHL groups coincided with human studies [34]. Moreover, [2] stated that serum hepatocyte-derived miRNA-122 is a highly stable blood indicator that has a diagnostic value for marking hepatocellular damage in dogs.

Conclusion

The current study assumed that miR-122, and IGF-1 in combination with ultrasound are valuable tools for the diagnosis of different hepatic affections in dogs.

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Funding statement

Not applicable

Declaration of Conflict of Interest

The authors have no conflicts of interest to declare.

TABLE 1. Oligonucleotide primers and probes used in SYBR Green real-time PCR.

Gene	Primer sequence (5'-3')	Reference
U6 (housekeeping)	GCTTCGGCAGCACATATACTAAAAT	**[15]**
	CGCTTCACGAATTTGCGTGTCAT	
MiRNA-122	Gcgagcacagaattaatacgac	**[3]**
	Tggagtgtgacaatggtgtttg	

TABLE 2. Cycling conditions for SYBR green real-time PCR according to Quantitect SYBR green PCR kit.

Gene	Reverse transcription	Primary Denaturation	Amplification (40 cycles)			Dissociation curve (1 cycle)		
			Secondary denaturation	Annealing (Optics on)	Extension	Secondary denaturation	Annealing	Final denaturation
U6 (housekeeping)	50˚C 30 min.	94˚C 15 min.	94˚C 15 sec.	60˚C 30 sec.	72˚C 30 sec.	94˚C 1 min.	60˚C 1 min.	94˚C 1 min.
MiRNA-122	50˚C 30 min.	94˚C 15 min.	94˚C 15 sec.	55˚C 30 sec.	72˚C 30 sec.	94˚C 1 min.	55˚C 1 min.	94˚C 1 min.

TABLE 3. Clinical examination of different group of liver diseases in dogs

Parameter	Control (n=9)	Acute Hepatitis (n=9)	Chronic Hepatitis (n=9)	Cholangitis (n=9)	*P-Value*
Temperature (C°)	38.62^{b}±0.1	39.42^{a} ± 0.17	38.64^{b} ± 0.1	38.52^{b} ± 0.08	<0.001
Pulse rate (beat/min.)	107^{b} ± 5.38	134.6^{a} ± 3.55	112.6^{b} ± 3.6	112^{b} ± 4.6	<0.001
Respiratory rate (breath/min.)	29^{ab} ± 2.9	40^{a} ±3.53	25^{b} ± 3.5	28.4^{ab} ± 2.65	<0.001

Data are presented as (Mean ± SE). S.E = Standard error. Mean values with different superscript letters in the same row are significantly different at (P<0.001).

TABLE 4. Hematological alterations in different groups of liver diseases in dogs

Hematological parameter	Control(n=9)	Acute Hepatitis(n=9)	Chronic Hepatitis(n=9)	Cholangitis(n=9)	*P-Value*
RBCs (10^6/ul)	7.45^{a}± 0.24	5.17^{b} ± 0.39	2.19^{c} ± 0.46	6.60^{ab} ± 0.27	<0.001
Hb (g/dl)	14.8^{a} ± 0.28	10.92^{a} ± 1.12	5.32^{b} ± 1.5	14.72^{a} ± 0.59	<0.001
PCV%	45.6^{a} ± 0.5	32.86^{b} ±1.6	30.88^{b} ± 5.45	42.9^{ab} ± 0.71	<0.001
WBCs (10^3/ µl)	11.72^{c} ± 0.3	30.59^{b} ± 2.47	17.88^{a}± 4.8	15.17^{bc} ± 8.14	<0.001
Neutrophil (10^3/µl)	8.16^{b}±0.19	25.98^{a}±5.2	12.8^{ab}±2.9	11.58^{ab}±4.34	<0.001
Lymphocyte (10^3/µl)	3.06^{a} ± 0.27	3.86^{a} ± 0.36	4.06^{a} ± 0.51	3.2^{a} ± 0.23	0.227
PLTs (10^3/ul)	113.2^{b} ± 13.3	114^{b} ± 34.64	328^{a} ± 54.3	109^{b} ± 12.07	<0.001
Eosinophil (10^3/µl)	0.04^{b}± 0.02	0.7^{b} ± 0.36	0.06^{b} ± 0.04	0.06^{b} ± 0.02	<0.001
Monocyte (10^3/µl)	0.44^{b} ± 0.02	0.2^{b} ± 0.05	0.96^{b} ± 0.18	0.36^{b} ± 0.09	<0.001
MCV (fL)	62.94^{ab} ± 0.34	64.02^{ab} ± 1.83	56.36^{b} ± 5.49	64.12^{ab} ± 0.77	<0.001
MCH (pg)	20.58^{ab} ± 0.05	21.36^{ab} ± 1.05	17.44^{b} ± 2.02	20.34^{ab} ± 0.18	<0.001
MCHC(g/dl)	32.44^{a} ± 0.33	33.34^{a} ± 0.79	30.58^{a} ± 1.17	30.42^{a} ± 0.58	0.227

Data are presented as (Mean ± SE). S.E = Standard error. Mean values with different superscript letters in the same row are significantly different at (P<0.001)

TABLE 5. Biochemical alterations in different groups of liver disease in dogs

Biochemical parameter	Control(n=9)	Acute Hepatitis(n=9)	Chronic Hepatitis(n=9)	Cholangitis (n=9)	*P-Value*
ALT(U/L)	30.35^{c} ± 3.9	189.57^{b} ± 56.3	89.62^{bc} ± 9.9	26.46^{c} ± 3.26	<0.001
AST(U/L)	28.12^{c} ± 1.39	195.1^{b} ± 44.1	62.5^{c} ± 3.9	39.8^{c} ± 1.42	<0.001
Creatinine(mg/dl)	0.58^{c} ± 0.07	0.71^{bc} ± 0.12	1.16^{a} ± 0.03	0.82^{bc} ± 0.08	<0.001
Urea(mg/dl)	10.34^{c} ± 2.5	30.59^{bc} ± 2.47	82.19^{a} ± 4.8	33.17^{b} ± 8.14	<0.001
Cholesterol(mg/dl)	182^{a} ± 27	243.6^{a} ± 18.4	214.56^{a} ± 33.4	178.03^{a} ± 22.9	0.227
Albumin(g/dl)	2.3^{a} ± 0.19	2.0^{ab} ± 0.28	1.2^{b} ± 0.05	2.21^{a} ± 0.23	<0.007
Triglycerides(mg/dl)	37.47^{a} ± 6.1	53.03^{a} ± 14.24	50.8^{a} ± 3.09	40.11^{a} ± 8.9	0.036
MDA (nmol/ml)	15.07^{c} ± 0.95	89.86^{ab} ± 4.02	77.5^{b} ± 3.3	23.85^{c} ± 1.8	<0.001
SOD (U/gHb)	11.82^{a} ± 0.96	4.01^{b} ± 0.3	6.3^{b} ± 0.51	11.55^{a} ± 1.1	<0.001
GSH (U/gHb)	14.5^{a} ± 1.38	2.3^{b} ± 0.09	5.16^{b} ± 0.74	14.5^{a} ± 1.38	<0.001
IL6 (pg/ml)	1.48^{c} ± 0.11	9.2^{a} ± 0.49	5.9^{b} ± 0.72	2.23^{c} ± 0.17	<0.001
CRP (mg/dL)	2.97^{c} ± 0.29	46.9^{a} ± 3.74	15.7^{b} ± 1.39	5.65^{c} ± 0.58	<0.001
Total protein(g/dl)	7.29^{a} ± 0.42	4.22^{c} ± 0.24	5.47^{b} ± 0.1	3^{d} ± 0.08	<0.001
Total bilirubin(mg/dl)	0.68^{d} ± 0.12	2.93^{a} ± 0.2	1.8^{bc} ± 0.28	1.6^{c} ± 0.08	<0.001
ALP (u/L)	86.02^{c} ± 1.67	269.9^{a} ± 9.7	185.7^{b} ± 7.2	88.34^{c} ± 5.7	<0.001
Direct bilirubin (mg/dl)	0.34^{b} ± 0.08	0.63^{b} ± 0.08	0.54^{b} ± 0.07	1.39^{a} ± 0.23	<0.001
IGF1 (ng/ml)	390.1^{a} ± 6.4	95^{c} ± 2.8	306.8^{ab} ± 6.7	295.9^{b} ± 45.5	<0.001

Data are presented as (Mean ± SE). S.E = Standard error. Mean values with different superscript letters in the same row are significantly different at (P<0.001).

TABLE 6. Pearson correlation coefficient between biochemical parameters and diagnostic biomarkers of hepatic disease

	ALT	AST	Chol.	Albumin	Triglycerides	MDA	SOD	GSH	IL6	CRP	TP	TB	ALP	DB	IGF I
ALT	1	0.852**	0.3700	-0.301	-.047	0.798**	-.715**	-.698**	0.589**	0.574**	-.459*	0.120	0.691**	0.398*	-.717**
AST		1	0.353	-.363	0.082	0.793**	-.712**	-.655**	0.624**	0.577**	-.573**	0.17	0.700**	0.473*	-.763**
Cholesterol			1	0.165	0.02	0.382	-.269	-.440	0.491*	0.451*	-.184	-.048	0.507*	-.085	-.430
Albumin				1	-.117-	-.588-**	.426*	.478*	-.347-	-.065-	.286	.127	-.433-*	-.114-	.348
Triglycerides					1	-.102-	.104	.137	-.186-	-.166-	-.661-**	-.432-*	-.138-	.553**	-.190-
MDA						1	-.874-**	-.865-**	.861**	.665**	-.497*	-.144-	.918**	.184	-.770**
SOD							1	.897**	-.835**	-.728**	.366	.117	-.866**	-.168	.743**
GSH-Px								1	-.809**	-.753**	.307	.146	-.882**	.000	.673**
IL6									1	.847**	-.372	-.215	.939**	.021	-.789**
CRP										1	-.269	-.182	.836**	-.052	-.779**
Total protein											1	.495*	-.397*	-.666**	.626**
Total bilirubin												1	-.153	-.108	.184
ALP													1	-.028	-.820**
Direct bilirubin														1	-.297
ILGFI															1

**. Correlation is significant at the 0.01 level. *. Correlation is significant at the 0.05 level.

TABLE 7. Serum hepatocyte-derived miRNA 122 analysis

Group	U6 CT	MiRNA-122	Δ CT	Δ Δ CT	Fold change
Control	19.41	22.12	0	0	0
Acute hepatitis	20.02	19.40	-0.61	2.72	10.0561
Chronic hepatitis	19.39	19.26	0.02	2.86	7.1602
Cholangitis	18.50	20.04	0.91	2.08	2.2501

miRNA = microRNAs, U6 =spliceosomal, CT = Cycle threshold

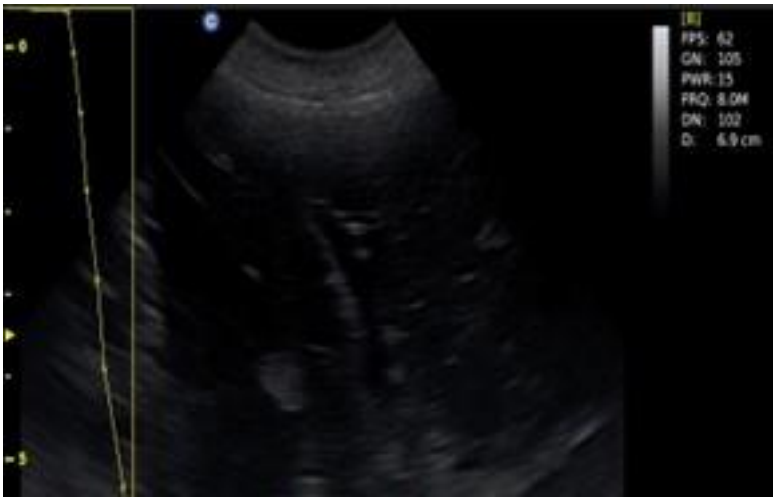

Fig. 1. Ultrasonographic image of the liver showing hypoechogenicity of the liver tissue in dogs affected with acute hepatitis.

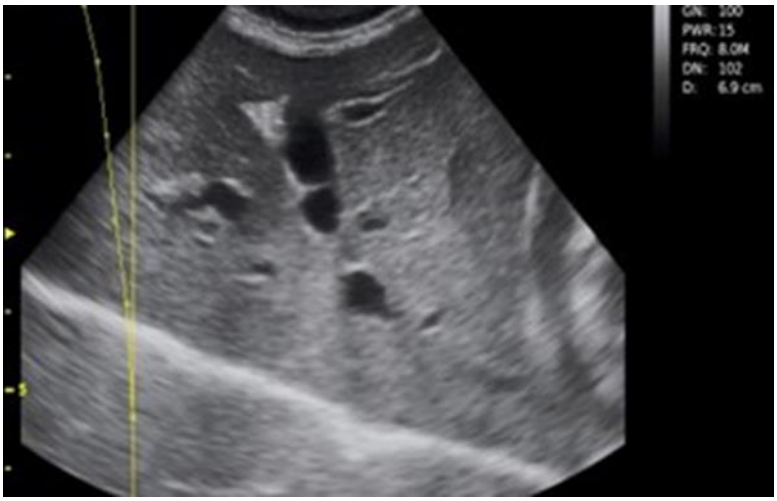

Fig.2. Ultrasonographic image of the liver showing diffuse hyperechogenicity of liver parenchyma in dogs affected with chronic hepatitis.

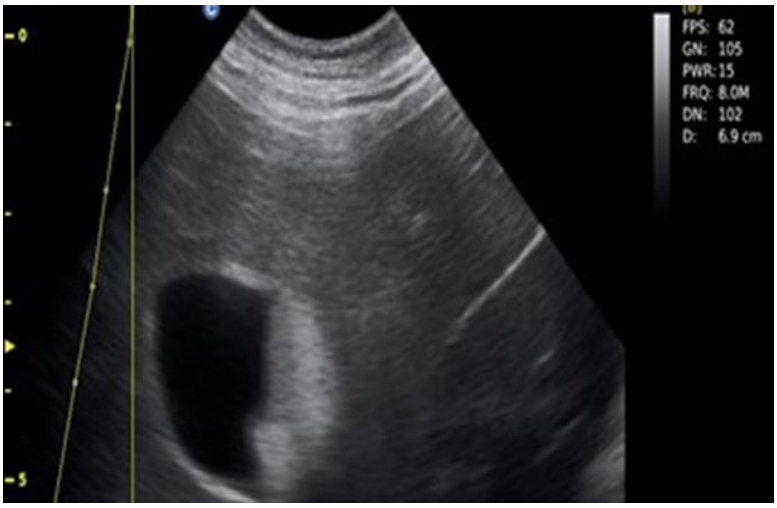

Fig.3. Ultrasonographic image of gall bladder showing that gall bladder contains echogenic material consistent with biliary sludge with thickening in the wall of the gall bladder.

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تقييم MiRNA-122 المشتق من خلايا الكبد وعامل النمو الشبيه بالانسولين ١ لتشخيص امراض الكبد في الكلاب

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الملخص

تهدف هذه الدراسة إلى تقييم miRNA-122 المشتق من خلايا الكبد وعامل النمو الشبيه بالأنسولين ١ (IGF-1) لتشخيص أمراض الكبد المختلفة في الكلاب. أجريت الدراسة على ١٢٢ كلباً من سلالات وأجناس وأعمار مختلفة، تم تقسيمها إلى ٤ مجموعات: التهاب الكبد الحاد(AH) ، التهاب الكبد المزمن(CH) ، التهاب الأقنية الصفراوية(CHL) ، ومجموعة السيطرة .(C) خضعت جميع الكلاب للفحوصات السريرية والدموية والكيميائية الحيوية والموجات فوق الصوتية. أظهرت الكلاب المريضة علامات سريرية مختلفة بما في ذلك اليرقان والحمى في مجموعة التهاب الكبد الحاد ، وفقدان الوزن، والبلادة، والاستسقاء في مجموعه التهاب الكبد المزمن، وانخفاض كبير ($P \geq 0.001$) في عدد كرات الدم الحمراء ومحتوى هيموجلوبين في مجموعة التهاب الكبد المزمن، وارتفاع كبير ($P \geq 0.001$) لـ ALT ، AST، IL6، MDA، CRP، وانخفاض ملحوظ ($P \geq 0.001$) في الألبومين، SOD، GSHفي مجموعات التهاب الكبد الحاد والمزمن .أظهر IGF-1 انخفاضًا كبيرًا في مجموعة التهاب الكبد الحاد. كشف تحليل miRNA-122 عن زيادة كبيرة في مجموعات التهاب الكبد الحاد، المزمن، و التهاب الاقنية الصفراوية. كشفت نتائج الفحص بالموجات فوق الصوتية عن زيادة منتشرة في صدى انسجه الكبد في مجموعة التهاب الكبد المزمن وانخفاض منتشر في صدى انسجة الكبد في مجموعة التهاب الكبد الحاد. خلصت الدراسة الحالية إلى أن miRNA-122 المشتق من خلايا الكبد هو علامة حيوية جزيئية حساسة لتشخيص أمراض الكبد المختلفة في الكلاب. بالإضافة إلى ذلك، يعد IGF-1 علامة حيوية مفيدة لتشخيص مجموعات التهاب الكبد الحاد والمزمن .

الكلمات الدالة: الكلاب ، الكبد ، miRNA-122 المشتق من خلايا الكبد ، عامل النمو الشبيه بالانسولين ١ ، الموجات فوق الصوتية.