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Original article

Suitability of using serum hialuronic acid concentrations in the diagnosis of canine liver fibrosis

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Abstract

There are multiple dynamic changes associated with the metabolism of the extracellular matrix (ECM) which occur in the course of liver fibrosis. Therefore, the evaluation of parameters reflecting the deposition of ECM, the activity of myofibroblasts and the synthesis and degradation of collagen may aid in the diagnosis of liver fibrosis. Hyaluronic acid is considered to be a marker of ECM deposition. It is a glycosaminoglycan synthesized by hepatic stellate cells and degraded by hepatic sinusoidal endothelial cells. The aim of this study was to evaluate the concentration of hyaluronic acid in dogs with different degrees of liver fibrosis. The study was carried out on 29 dogs with liver disease. A core needle biopsy of the liver was performed in order to assess the degree of hepatic inflammation. Then, hyaluronic acid serum concentrations were measured. The dogs were divided into five groups based on the histopathological examination and the evaluation of the degree of hepatic fibrosis. The study showed that serum hyaluronic acid concentrations were low in patients with first stage liver fibrosis and in controls, while they were twice as high as control values in the group of dogs with second stage liver fibrosis. These concentrations were three-fold greater than control values in patients with third stage liver fibrosis, and seven-fold greater in patients with liver cirrhosis. Based on the results, it was concluded that serum hyaluronic acid is a useful marker of liver fibrosis and may aid in determining the degree of its advancement.

Key words: liver inflammation, dog, hyaluronic acid, liver fibrosis

Introduction

Liver fibrosis is caused by a disruption of the synthesis and degradation of the cellular matrix components. This leads to excessive connective tissue overgrowth in the liver, the formation of connective tissue partitions, a disruption of its physiological architecture and the formation of regenerative nodules (Błazik and Durlik 2010, Pinzani and Macias-Barragan 2010, Błaszczuk at al. 2012). Liver fibrosis is a polyethologic disorder. It is most often caused by long term exposure of the liver tissue to toxic agents,

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by metabolic disorders, biliary tract disease and the damaging effects of certain drugs.

Both invasive and non-invasive methods are used to diagnose liver fibrosis. The diagnosis is usually made based on blood test results, diagnostic imaging and a liver biopsy (Afdhal 2004). The current most common method used to assess the degree of liver damage is a core needle biopsy. Despite the diagnostic value of this type of biopsy, it has several limitations associated with the risk of complications, such as hemorrhage, pneumothorax and shock (Friedman 2008). This method is also prone to errors, such as collecting an inadequate sample or collecting a sample from an area not affected by any pathological lesions. In human medicine, there have been numerous studies assessing the use of noninvasive markers of fibrosis to diagnose liver disease (Guechot at al. 1996, Guechot at al. 2000, Gutkowski 2007, Błazik and Durlik 2010, Fernandez-Varo and Jimenz 2011). Serum hyaluronic acid (HA) was found to be a very useful parameter in the assessment of the severity of liver fibrosis (Laurent 1992). Several studies carried out in humans have confirmed its clinical efficacy. This prompted us to determine the hyaluronic acid serum concentrations in dogs with a varying degree of liver fibrosis.

Materials and Methods

The study was carried out on 29 dogs of various breeds (12 mixed breed dogs, 5 Labrador retrievers, 4 cocker spaniels, 3 German shepherds, 2 boxers, 1 Staffordshire bull terrier, 1 miniature poodle and 1 Yorkshire terrier) and of both genders (12 males, 17 females) between 5 and 16 years old. All dogs were suspected of having chronic liver parenchyma damage and were referred to the Clinic of the Department of Internal Diseases with Clinic of Horses, Dogs and Cats for a liver biopsy. Prior to this procedure, blood samples were obtained from all the dogs and basic blood biochemistry was carried out (AST, ALT, ALP, GGT, total bilirubin). Additionally, EDTA anticoagulated blood was centrifuged and stored at -20°C. Serum HA levels were measured using the Corgenixe Hyaluronic Acid (HA) quantitative Test Kit (Corgenix, Inc., Broomfield, Co, USA).

The core needle biopsy was carried out in all the animals using Tru-Cut needles. The biopsies were performed under general anesthesia. Xylazine (1 mg/kg.) and atropine (0.04 mg/kg) were administered intramuscularly to premedicate each animal. Anesthesia was induced using propofol (2-6 mg/kg) administered intravenously. The site of insertion of the needle biopsy was shaved and twice disinfected with alcohol. The procedure was performed under ultrasound guidance using an Aloka 3500 prosound ultrasound. Two biopsies were taken from the lesioned sites and the specimens were subsequently fixed in a 10% buffered formalin solution.

After being fixed, the liver samples were embedded in paraffin and sectioned into slices 4-6 μm thick. The specimens were then stained using hematoxylin and eosin (H-E), the Van Gieson method, and the Mallory method respectively and using staining for the presence of reticular fibers. The liver was assessed in terms of inflammation and fibrosis.

A 4 point scale approved by the Hepatology Group of the Polish Society was used to assess hepatic inflammation:

Grade 0 – no changes in the portal spaces

Grade 1 – minimal inflammation – scarce inflammatory infiltrates in portal spaces, little centrilobular inflammation, hepatocyte laminae intact,

Grade 2 – moderate inflammation – moderate inflammation in portal spaces, single sites of piece meal necrosis in hepatic lobules,

Grade 3 – marked inflammation – piece meal necrosis affecting a minority of the laminar circumference in all portal tracts, marked inflammatory and necrotic activity within the lobules,

Grade 4 – extensive inflammation – piece meal necrosis affecting the majority of the laminar circumference, large centrilobular inflammation with bridging necrosis (Goodman 2007).

A 4 stage scale according to Scheuer was applied to assess liver fibrosis:

Stage 0 (F0) – normal – no fibrosis, single collagen fibers in portal spaces

Stage 1 (F1) – fibrosis within portal spaces with an extension of the portal tracts

Stage 2 (F2) – peri-portal fibrosis and possibly single bridging fibrosis while maintaing lobular structure,

Stage 3 (F3) – the presence of multiple fiber spans, destruction of the lobular architecture, no regeneration reaction.

Stage 4 (F4) – disseminated fibrosis or cirrhosis (Goodman 2007).

Having adopted this classification, we were able to assess the concentration of hyaluronic acid depending on the degree of changes in the liver tissue.

The dogs were divided into five groups based on the degree of liver fibrosis:

– group 1, comprising 6 dogs, which did not have liver fibrosis (F0),

– group 2, comprising 5 dogs, who had first degree liver fibrosis (F1),

– group 3, comprising 5 dogs, who had second degree liver fibrosis (F2),

Table 1. Results of chosen biochemical parameters as well as degree of liver inflammation and fibrosis based on canine biochemical blood results.

	AST [U/l]	ALT [U/l]	ALP [U/l]	GGT [U/l]	Total bilirubin [mmol/l]	Inflammation	Fibrosis
1	234	411	242	2	76	2	4
2	311	1187	442	29	56	1	4
3	78	54	170	5	1.8	0	0
4	171	574	96	8	3.3	1	2
5	32	77	170	1	1.8	1	0
6	117	205	160	18	1	0	1
7	96	871	199	6	4.3	0	1
8	81	632	61	13	3	1	1
9	24	60	78	3	2.8	0	0
10	52	24	41	4	1.8	1	0
11	43	213	56	1	1.8	1	1
12	218	388	320	20	7	1	2
13	233	1314	452	16	32	3	4
14	160	587	382	13	17	2	3
15	320	715	438	20	24	2	4
16	27	214	543	21	11	1	3
17	338	1807	103	15	14	2	4
18	31	53	86	2	2.6	0	0
19	40	356	542	10	3.2	0	1
20	56	138	287	12	2	0	0
21	214	412	214	10	1	1	2
22	226	1668	170	33	12	3	4
23	47	117	468	2	8.9	1	3
24	234	411	242	2	23	2	4
25	43	34	43	1	2.6	0	2
26	267	964	488	29	16	3	4
27	107	1111	742	188	9.2	1	3
28	64	30	74	13	2	1	2
29	198	277	411	27	5.4	2	3

– group 4, comprising 5 dogs, who had third degree liver fibrosis (F3),

– group 5, comprising 8 dogs, who had fourth degree liver fibrosis (F4).

Statystical analysis was performed using STATISTICA version 10 (StatSoft Inc., Poland). Data are expressed as median. Correlation between HA and blood markers of liver function were determined using Speerman correlation. A p-value of = 0.05 was considered statistically significant.

Results

The results of some of the biochemical parameters as well as the degree of liver inflammation and fibrosis are presented in Table 1.

Based on the histopathological examination of the liver specimens, eight dogs (27.5%) had no liver inflammation, twelve dogs (41.3%) were found to have grade 1 inflammation, six dogs (20.6%) had grade 2 inflammation, and three dogs (10.3%) had grade 3 inflammation. Grade 4 inflammation was not present in any dog.

The mean HA serum concentration amounted to 51.12 ± 21.13 ng/ml in dogs that had no liver inflammation, 96.3 ± 42.9 ng/ml in dogs with grade 1 inflammation, 264.94 ± 137.88 ng/ml in dogs with grade 2 inflammation and 487.82 ± 150.31 ng/ml in dogs with grade 3 inflammation.

A graphic presentation of the HA serum concentrations in conjunction with the stage of liver inflammation is presented in Fig. 1.

Six dogs (20.6%) had no liver fibrosis, five had first degree fibrosis (17.2%), five had second and third degree fibrosis (17.2%) and eight had cirrhosis (27.5%).

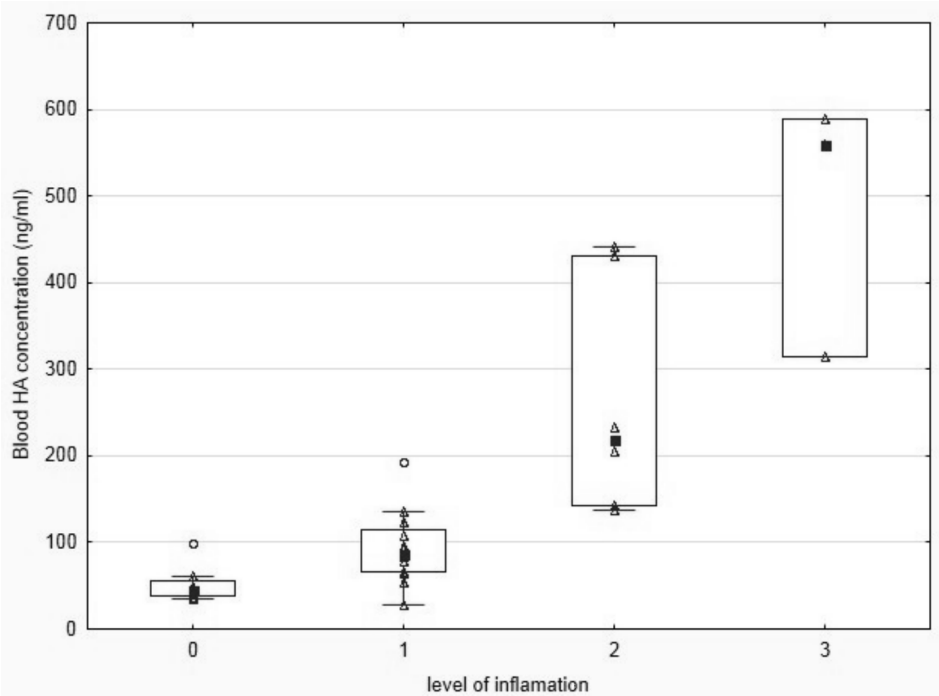

Fig 1. Box plots of blood HA concentrations according to stage of inflammation.

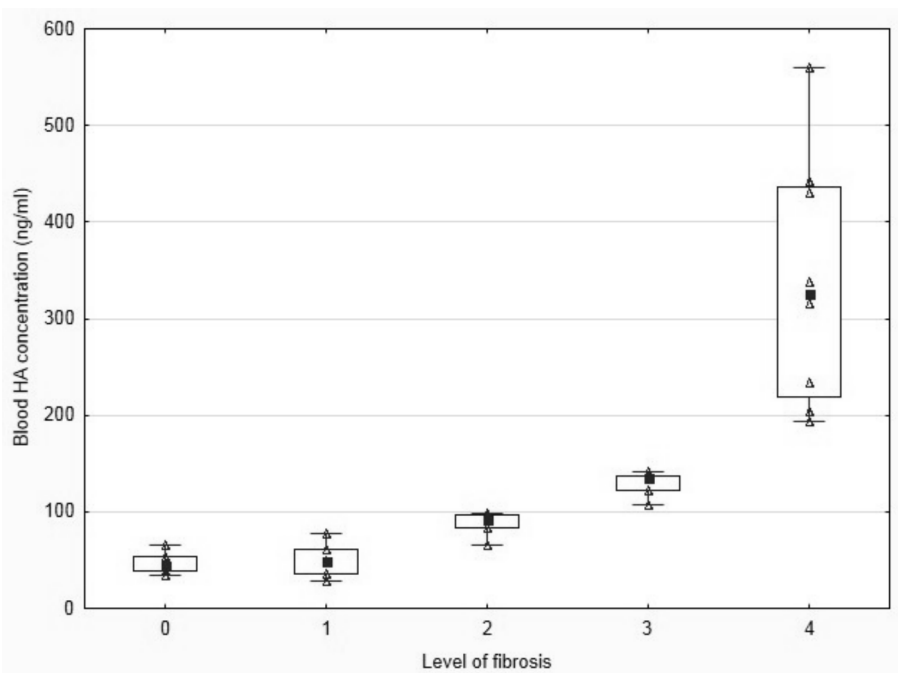

Fig 2. Box plots of blood HA concentrations according to stage of fibrosis.

The mean HA serum concentrations were 47.12 ± 11,04 ng/ml in group 1, 50.47 ± 19.76 ng/ml in group 2, 87.36 ± 13.16 ng/ml in group 3 and 128.84 ± 13.72 ng/ml in group 4. The HA serum concentration reached 370.90 ± 157.26 ng/ml in eight dogs, which were diagnosed with fourth degree fibrosis based on the biopsy result.

A graphic presentation of the HA serum concentrations in conjunction with the stage of liver fibrosis is presented in Fig. 2.

Discussion

In recent years, numerous studies evaluating the usefulness of serum markers to diagnose liver fibrosis non-invasively have been conducted in human medicine (Guechot at al. 1995, Guechot at al. 2000, Gutkowski 2007, Błazik and Durlik 2010, Fernandez-Varo and Jimenz 2011). These studies analyzed direct and indirect markers aiming to find a marker that could effectively detect liver fibrosis and determine the degree of its advancement (Baszczuk at al. 2012). Indirect markers enable an evaluation of liver dysfunction, while direct markers reflect the metabolism of the extracellular matrix (EMC) (Gutkowski at al. 2007, Baranowa at al. 2011).

Many dynamic changes that occur in the course of liver fibrosis are associated with ECM metabolism. Therefore, analysing parameters that reflect the process of depositing components of the ECM, the activity of myofibroblasts, and the synthesis and degradation of collagen is useful. Direct markers of liver fibrosis can be divided into 3 groups: markers associated with excessive EMC deposition, markers associated with EMC degradation, and cytokines and chemokines closely associated with liver fibrosis (Gutkowski at al. 2007).

The first group of markers includes the propeptide of type I procollagen (PICP), the procollagen III amino-peptide, laminine, chondrex and hyaluronic acid. The second group contains metalloproteinase (MMPs), which are proteolytic enzymes. Studies have found that MMP-2 and MMP-9 are of particular importance in the diagnosis of liver fibrosis (Gutkowski at al. 2007). Cytokines and chemokines related to liver fibrosis make up the third group, including transforming growth factor α – TGF-α, transforming growth factor β – TGF-β and the platelet derived growth factor -PDGF (Baszczuk at al. 2012).

The current study attempted to evaluate the concentration of hyaluronic acid in dogs with a varying degree of liver damage. Hyaluronic acid is a glycosaminoglycan synthetized by hepatic stellate cells and degraded by sinusoidal endothelial cells (Laurent and Fraser 1992). Studies carried out in humans have shown that the serum concentration of hyaluronic acid in patients with liver disease reflects the severity of liver fibrosis, is a useful indicator of cirrhosis and positively correlates with the degree of hepatitis (Guechota at al. 1995, Guechota at al. 1996, Körner at al. 1996). In 1999, a group of scientists from Japan sought to evaluate hyaluronic acid concentrations in dogs with cirrhosis (Kenemoto at al. 2009). They measured serum hyaluronic acid concentrations in two groups of dogs – ones with liver disease without cirrhosis, and those with cirrhosis. They demonstrated that the mean hyaluronic acid concentration in healthy dogs amounted to 73 μg/l, 153 μg/l in dogs with parenchymal damage without cirrhosis and 500 μg/l in dogs with cirrhosis. Our study, on the other hand, attempted to assess the HA concentration in dogs with varying degrees of liver fibrosis, and through this the diagnostic value of HA in fibrosis. The concentrations of HA in healthy patients (F0) and in those with first stage fibrosis (F1) were low, while those from patients with second stage fibrosis were two-fold larger than in controls. Animals with third stage fibrosis (F3) had three-fold larger concentrations, while those with fourth stage fibrosis (F4) had seven-fold greater concentrations than controls. Additionally, we found a positive correlation between the degree of inflammation and the serum concentration of hyaluronic acid, which is in accordance with the findings of Guechota et al. carried out in humans.

The following conclusions were drawn from the present study:

– Hyaluronic acid concentrations correlate with the degree of liver fibrosis.

– The evaluation of the concentration of hyaluronic acid is a useful serum marker of liver fibrosis.

– The concentration of hyaluronic acid increases in the course of hepatic inflammation, which renders it a good indicator of liver inflammation.

In summary, hyaluronic acid is a serum marker that may have wide use in the non-invasive diagnosis of liver disease. However, in order to assess its usefulness, a study on a larger group of animals with various liver pathologies is required.

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