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Research article

Evaluation of Glypican-3 mRNA and Alpha-fetoprotein mRNA as biomarker for hepatocellular carcinoma in Dogs: A preliminary study

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Abstract

Glypican-3 (GPC-3) and alpha-fetoprotein (AFP) are considered powerful biomarkers for human hepatocellular carcinoma (HCC). The elevation of GPC-3 and AFP gene expression is commonly found in individual HCC patients. The detection of these biomarkers can be achieved with the examination of blood or tissue samples. Moreover, mRNA expressing GPC-3 and AFP are evaluated for detecting HCC in patients. In veterinary practice, data on GPC-3 and AFP of gene expression remain limited. Therefore, this study aims to evaluate the GPC-3 and AFP gene expression in dogs with HCC compared with other hepatic diseases. Twenty dogs were divided into two groups: HCC (n=6), and non-HCC (n=6). The liver tissue and plasma samples of each group were examined for GPC-3 and AFP gene expression by qPCR. The results showed a no statistical difference in the expression levels of GPC-3 gene in liver and plasma samples of dogs with HCC compared with non-HCC dogs. However, there was a positive correlation between the expression of GPC-3 gene and levels of aspartate transaminase (AST) in the HCC group (*p-value* = 0.002, *R* = 0.967). Moreover, the sensitivity, specificity, and accuracy of GPC-3 gene expression were higher than AFP gene expression in the HCC group. Therefore, this study conveys the expression of GPC-3 gene and AFP gene, suggesting a potential association with the development of HCC in dogs. The findings, particularly regarding the GPC-3 gene, could contribute to improved diagnostic methods in the future.

Keywords: Alpha-fetoprotein, Dogs, Glypican-3, Hepatocellular carcinoma.

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INTRODUCTION

Canine hepatocellular carcinoma (HCC) is a sporadic disease accounting for 0.6–1.3% of all canine tumors (Liptak et al., 2004a). Several studies have revealed that HCC usually affects geriatric male dogs. Purebred dogs, especially Shih Tzus, Yorkshire Terriers, Beagles, Poodles, Golden Retrievers, and Labrador Retrievers, are overrepresented (Liptak et al., 2004b; Masserdotti and Drigo, 2012; Leela-Arporn et al., 2019a). Furthermore, dogs with hyperadrenocorticism have a high risk of HCC (Leela-arporn, 2019; Leela-Arporn et al., 2019a).

The clinical signs of dogs with HCC are non-specific. However, lethargy, anorexia, vomiting, diarrhea, and abdominal enlargement are common signs (Patnaik et al., 1981; Liptak et al., 2004a; Teshima et al., 2013). Elevated hepatobiliary enzymes, especially alanine aminotransferase (ALT), AST, and alkaline phosphatase (ALP), are commonly detected (Liptak et al., 2004b; Leela-Arporn et al., 2019a; Leela-Arporn et al., 2019b). Furthermore, imaging diagnosis is the first investigator to recognize hepatic masses. According to its ultrasonographic characteristics, HCC can be classified into massive, nodular, and diffuse patterns, with liver biopsy and pathological examination being the definitive diagnostic method (Patnaik et al., 1981; Balkman, 2009). According to WHO criteria, HCC can be histologically classified into four grades: well-differentiated, moderately-differentiated, poorly-differentiated, and undifferentiated (Nagtegaal et al., 2020). Dogs with HCC usually visit the hospital with signs of hepatic failure, including ascites, hypoglycemia, hypoalbuminemia, severe anemia, and coagulopathies (Moyer et al., 2021; Lapsley et al., 2022). These signs interfere with liver biopsy procedures. Therefore, non-invasive diagnoses seem to be an essential tool to investigate these complicated cases.

Alpha fetoprotein (AFP) is a well-known marker that has been used to diagnose complicated HCC and non-identified HCC cases in both humans and dogs (De Las Mulas et al., 1995). It is an oncofetal protein that overexpresses in dogs with HCC (Hahn and Richardson, 1995). AFP is not only found in HCC tissue but also in serum (Kitao et al., 2006). A previous study demonstrated that the AFP gene expressions in blood and liver tissues correlate with canine HCC (Yamada et al., 1999). However, this marker is also expressed in other hepatobiliary diseases such as fatty degeneration hepatopathy, glycogen accumulated hepatopathy, chronic hepatitis, nodular hyperplasia, and hepatocellular adenoma (Yamada et al., 1999). Therefore, it is not a specific marker of HCC in dogs.

Glypican-3 (GPC-3) is a cell surface proteoglycan protein that expresses particularly in the yolk sac and fetal liver during embryonic development (Nakatsura et al., 2003). In general, all forms of GPC-3 will be downregulated in healthy adult humans (Nakatsura et al., 2004). GPC-3 is involved in cell proliferation pathways, tumor migration, and the tumor microenvironment of HCC tissues (Pan et al., 2013; Wang et al., 2018; Kolluri and Ho, 2019). Therefore, GPC-3 protein has been used as a specific novel marker for HCC diagnosis in humans (Kolluri and Ho, 2019). Interestingly, re-expression of GPC-3 protein in HCC patients is significantly related to HCC recurrence (Li et al., 2006). Several studies have demonstrated that the specificity of GPC-3 is higher and more sensitive than AFP (Capurro et al., 2003; Filmus and Capurro, 2004; Shirakawa et al., 2009). Furthermore, in the early stage of HCC, the GPC-3 gene expression level seems to be greater than other biomarkers. In the case of AFP-negative HCC patients, GPC-3 gene expression is still detected (Abdelgawad et al., 2013; Tahon et al., 2019; Karaoğullarindan et al., 2022). Therefore, GPC-3 gene expression in blood and HCC tissue might be used as the highest potential marker to diagnose HCC in patients (Yao et al., 2013).

In veterinary medicine, there have been few studies describing GPC-3 expression in animals with hepatopathies. Only one report demonstrated the GPC-3 protein expression in the poor stage of HCC dogs by immunohistochemistry and mentioned that GPC-3 protein expression may be correlated with poor prognosis (van Sprundel et al., 2010). However, there are no reports regarding GPC-3 gene

expression in HCC dogs. This study, therefore, aims to evaluate the expression of GPC-3 gene in dogs with HCC and the potential development of GPC-3 gene expression level as a non-invasive diagnostic test for dogs with suspected HCC.

MATERIALS AND METHODS

Samples

Twenty hepatic tissue and plasma samples were obtained from dogs presenting at the Small Animals Hospital, Chiang Mai University, with a history of elevated liver enzymes and various types of hepatic lesions confirmed by ultrasound. All tissue samples were histologically classified into HCC (n=6), and non-HCC (n=6) groups. The liver samples were stabilized in RNAlater solution (Invitrogen, Carlsbad, CA, USA), with plasma samples stored at -80 °C prior to RNA extraction. The two normal liver and plasma was used for control. The research protocols were approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine, Chiang Mai University (S3/2566).

Individual information, including sex, age, breed, concurrent diseases, clinical presentation, hematological and blood chemical profiles, and histopathological results were recorded.

RNA isolation and quantitative PCR (qPCR)

In the RNA extraction process, 20–50 mg minced liver sample in RNAlater solution and 600 µL plasma samples were extracted using a Purelink™ RNA mini kit (Invitrogen). All RNA samples were eluted with 50 µL of RNase-free water (Invitrogen). The RNA concentration was estimated using the Beckmann Colter machine (Invitrogen). The ultraviolet absorbance of the samples was measured at 260 and 280 nm. The acceptable mRNA concentration was 1,000 ng/µL.

The qPCR was used to detect the expression of GPC-3 and AFP genes from the fresh liver tissue and plasma, with GAPDH (Bio-Rad, Hercules, CA, USA) as a housekeeping gene control. The GPC-3 primers were newly designed in this study. The sequences of primers are summarized in Table 1. Each reaction had a total volume of 20 µL consisting of 5 µL CAPITAL™ qPCR Green Master Mix (biotechrabbit, Berlin, Germany), 0.8 µL forward primer, 0.8 µL reverse primer, 2 µL cDNA template, and 11.4 µL RNase-free water. The conditions for the PCR reaction were 2 min at 95 °C, followed by 40 cycles of denaturation at 95 °C for 15 sec and annealing/extension at 60 °C for 30 sec. The qPCR assays were duplicated with the ABI Prism GeneAmp 7300 Sequence Detection System (Applied Biosystems, Foster, Canada). The expression of GPC-3 and AFP genes was evaluated using the fold change method (Livak and Schmittgen, 2001).

Table 1 Primer sequences

Primer*	Sequence	PCR product	Reference
F-AFP	5'-TGAAGAGGGAAGACATAACTG-3'	112 bp	Wang et al., 2011
R-AFP	5'-AGC- AGCCCAAAGAAGAAT-3'		
F-GPC-3	5' TAAAGACTGTGGCCGAATGC 3'	107 bp	XM_038449070.1
R-GPC-3	5' AGCCTTGCATGACCACATTG 3'		
Canine GAPDH Primer	PrimePCR™ SYBR Green Assay: GAPDH, Dog	-	Bio-Rad

* F = forward; B = backward; AFP = alpha-fetoprotein; GPC-3 = glyplican-3; GAPDH = glyceraldehyde 3-phosphate dehydrogenase

Statistical analysis

Statistical analyses were investigated using IBM® SPSS® Statistics Program Version 25 (IBM®, Armonk, NY, USA). All dogs were classified into HCC, and Non-HCC groups for comparing clinicopathological parameters, $\log_2$ of relative expression of GPC-3, and AFP genes.

All parameters revealed non-normal distributed data. Therefore, the continuous variables were analyzed by the Mann Witney U test and described as the mean ± standard deviation and boxplot graph. The categorical data were analyzed by chi-square test and shown as frequency and percentage.

Pearson correlation was used to describe the relationship between GPC-3 gene expression and clinicopathological parameters.

The predictive cut-off value of $\log_2$ of relative expression was estimated from the receiver operating characteristic (ROC) curve and sensitivity, specificity, positive predictive value (PPV), and negative predictive values (NPV), then performed by 2x2 contingency for the different markers. A *p-value* <0.05 was considered statistically significant for all statistical analyses.

RESULTS

Demography

Twenty dogs (seven females and five males) were included in this study. The average age was 12.5 years. The dog breeds included Mongrels (n=4, 33.3%), Shih Tzus (n=4, 33.3%), Beagles (n=2, 16.66%), Poodles (n=1, 8.33%), and Pomeranians (n=1, 8.33%). According to the histopathological characteristics, all dogs were categorized into the HCC group (n=6), and non-HCC group (n=6). The latter consisted of degenerative hepatopathies (n=4), chronic cholangiohepatitis (n=1), and nodular hyperplasia (n=1).

The signalment, clinical signs, and disease concurrence in each group are summarized in Table 2. The hematological and blood chemical profiles of all groups are summarized in Table 3. Only elevated ALP showed a statistically significant difference between HCC, and non-HCC groups (*p-value*=0.008). Elevated ALP of more than three times the normal range was found in the HCC group (100%). However, no statistical differences were observed among the other biochemical parameters.

GPC-3 gene and AFP gene expression

GPC-3 gene can be detected in HCC, and non-HCC dogs. The $\log_2$ of relative expression values of GPC-3 gene in liver samples of HCC, and non-HCC groups were -1.0168±0.114, and -1.5571±0.311, respectively. In plasma, the $\log_2$ of relative expression values of GPC-3 gene in HCC, and non-HCC groups were -9.99±0.39, and -12.29±0.4, respectively.

The $\log_2$ of relative expression values of GPC-3 gene in the liver sample exhibited no significant difference between HCC and non-HCC groups (Figure 1a). In plasma samples, there were also no significant results for the $\log_2$ of relative expression values in HCC, and non-HCC (Figure 1b).

Table 2 Demography and clinical signs of HCC, and non-HCC

Parameter	HCC (n=6)	Non-HCC (n=6)	*p-value*
Age (mean±SD)	13.5±0.54	11.5±1.64	0.180
Sex			0.558
Male (n (%))	2 (33.33)	3 (50)	
Female (n (%))	4 (66.67)	3 (50)	
Breed			0.406
Mongeal (n (%))	3 (50)	1 (16.6)	
Shi-Tzu (n (%))	1 (16.6)	3 (50)	
Poodle (n (%))	1 (16.6)	0 (0)	
Beagle (n (%))	1 (16.6)	1 (16.6)	
Pomeranian (n (%))	0 (0)	1 (16.6)	
Clinical signs			0.182
Asymptomatic (n (%))	0 (0)	3 (50)	
Systematic (n (%))	6 (100)	3 (50)	
Abdominal distension (n (%))	6 (100)	3 (50)	
Abdominal pain (n (%))	6 (100)	1(16.7)	
Inappatite (n (%))	6 (100)	3 (50)	
Vomiting (n (%))	3 (50)	1(16.7)	
Concurrent diseases			0.456
Hyperadrenocorticism (n (%))	2 (33.33)		
Atopy (n (%))	1 (16.67)	1 (16.67)	
No (n, (%))	3 (50)	4 (66.66)	

AFP gene can be detected from liver samples in HCC (4/6, 66.6%), and non-HCC (4/6, 66.6%) dogs. In plasma samples, AFP gene can be detected from samples in HCC (5/6, 83.3%), and non-HCC (5/6, 83.3%) dogs.

The $\log_2$ of relative expression values of AFP gene in liver samples of HCC, and non-HCC groups were -2.869±0.54, and -2.062±0.60, respectively. In plasma sample, the $\log_2$ of relative expression values of AFP gene of HCC, and non-HCC groups were -3.5751, and -3.686, respectively.

There were no significant results for the $\log_2$ of relative expression values of AFP gene in the liver samples from HCC, and non-HCC (Figure 1c). In plasma sample, the $\log_2$ of relative expression values of AFP gene from the HCC group and non-HCC exhibited also no statistical significance (Figure 1d).

The correlation analyses of AST showed a strong positive correlation with liver GPC-3 gene (R=0.967, *p-value=0.002*) and a moderate positive correlation with plasma GPC-3 gene (R=0.884, *p-value=0.019*) (Table 4). In addition, the correlation between liver GPC-3 gene and plasma GPC-3 gene expression levels was significant in the HCC group (*p-value*=0.049, 95% CI) (Figure 2), whereas other parameters revealed no correlation with GPC-3 gene expression.

Table 3 Clinicopathological parameters of HCC, and non-HCC

Parameter	HCC	Non-HCC	*p-value*
Hematocrit (%) (mean±SD)	39.33±7.47	43.16±4.46	0.307
Normal (n (%))	4 (66.70)	6 (100)	0.182
Anemia (n (%))	2 (33.3)	0 (0)	
White blood cell count (x 10^3/mL) (mean±SD)	25.39±21.58	13.47±5.37	0.734
Normal (n (%))	2 (33.33)	5 (83.33)	0.242
Leukocytosis (n (%))	4 (66.70)	1 (16.65)	
Platelets count (x 10^3/mL) (mean±SD)	434.83±251.05	467.66±161.68	0.468
Normal (n (%))	4 (66.70)	5 (83.33)	0.632
Thrombocytosis (n (%))	1 (16.65)	1 (16.65)	
Thrombocytopenia (n (%))	1 (16.65)	0 (0)	
AST (U/L) (mean±SD)	130±171.6	30±27.10	0.516
Normal (n (%))	1 (16.65)	4 (66.70)	0.124
Elevated ≤ 3 times (n (%))	3 (50)	1 (16.65)	
Elevated ≥ 3 times (n (%))	2 (33.33)	1 (16.65)	
ALT (U/L) (mean±SD)	1,523.23±2,715.89	125.66±144.97	0.518
Normal (n (%))	1 (16.65)	3 (50)	0.098
Elevated ≤ 3 times (n (%))	2 (33.33)	2 (33.33)	
Elevated ≥ 3 times (n (%))	3 (50)	1 (16.7)	
ALP (U/L) (mean±SD)	2,030±1,202.73	850±550.06	0.041
Normal (n, (%))	0 (0)	0 (0)	0.008
Elevated ≤ 3 times (n (%))	0 (0)	1 (16.7)	
Elevated ≥ 3 times (n (%))	6 (100)	5 (83.33)	
Total protein (g/dL) (mean±SD)	7.18±0.74	6.88±0.98	0.149
Normal (n, (%))	3 (50)	5 (83.33)	
Decreased (n, (%))	0 (0)	0 (0)	
Increased (n, (%))	3 (50)	1 (16.7)	
Albumin (g/dL) (mean±SD)	3.05±0.66	3.03±0.38	0.207
Normal (n, (%))	5 (83.3)	3 (50)	0.545
Hypoalbuminemia (n, (%))	1 (16.7)	3 (50)	
Hyperalbuminemia (n, (%))	0 (0)	0 (0)	
Glucose (mg/dL) (mean±SD)	109.2±1.05	103.40±11.08	0.333
Normal (n, (%))	6 (100)	6 (100)	1.000
Hypoglycemia (n, (%))	0 (0)	0 (0)	
Hyperglycemia (n, (%))	0 (0)	0 (0)	

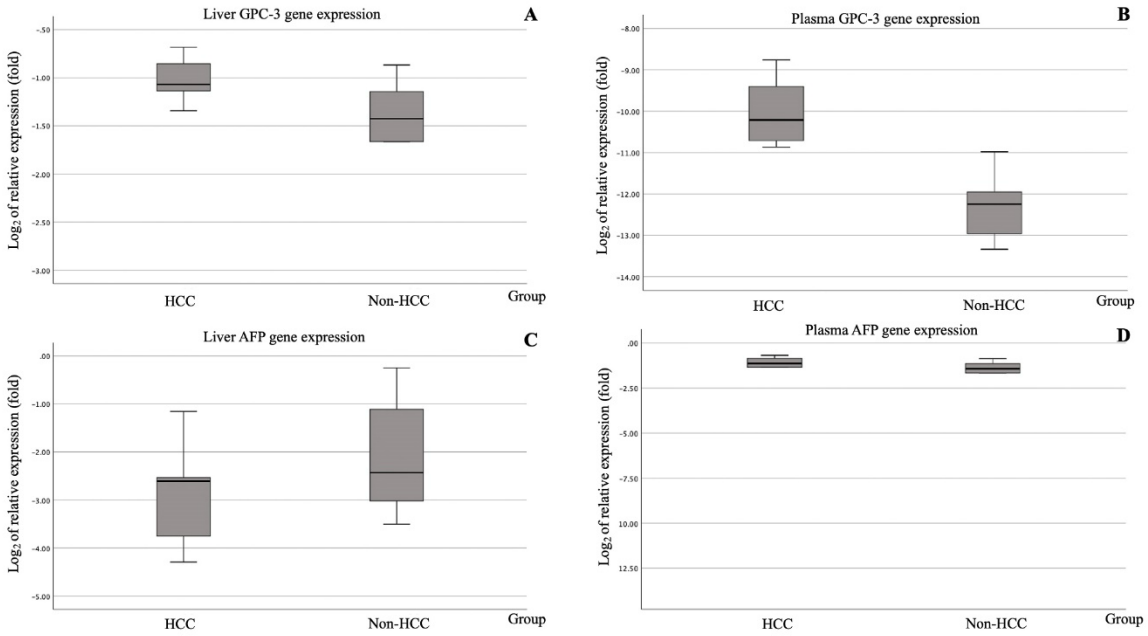

Figure 1 Comparison of expression levels of GPC-3 and AFP genes in HCC, and non-HCC groups. (A) Expression levels of GPC-3 gene from liver samples. Liver GPC-3 gene expression levels in HCC and non-HCC groups are no significant difference. (B) Expression levels of GPC-3 gene from plasma samples. The plasma GPC-3 gene expression levels in HCC, and non-HCC groups are no significant difference. (C) Expression levels of AFP gene from liver samples. Liver AFP gene expression levels in HCC, and non-HCC are no significant difference. (D) Expression levels of AFP gene from plasma samples. The plasma AFP gene expression levels in HCC and non-HCC groups are no significant difference.

Table 4 Correlation analyses of liver GPC-3 mRNA, plasma GPC-3 mRNA, and clinicopathological parameters in HCC group

Parameter	Liver GPC-3 mRNA		Plasma GPC-3 mRNA	
	R	*p-value*	*R*	*p-value*
Sex	0.375	0.464	0.23	0.662
Age	0.476	0.34	0.338	0.512
Hematocrit	0.516	0.294	0.219	0.677
WBC	-0.512	0.299	0.206	0.696
Platelets count	-0.157	0.299	0.206	0.696
AST	**0.967**	**0.002**	**0.884**	**0.019**
ALT	-0.069	0.896	0.199	0.706
ALP	0.036	0.946	0.107	0.841
Total protein	0.157	0.592	0.115	0.695
Albumin	0.259	0.62	0.079	0.887
Glucose	-0.09	0.769	0.171	0.576

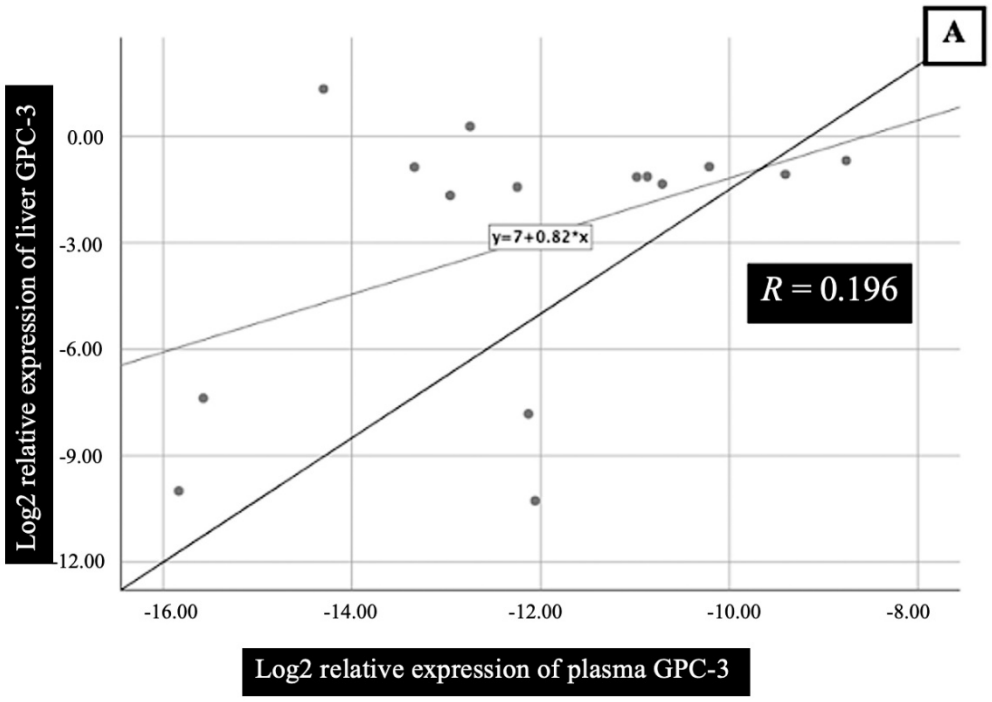

Figure 2 Correlation graph of log_2 of relative expression of GPC-3 gene in HCC group. (A) Correlation graph of log_2 of relative expression of GPC-3 gene from liver and plasma, shows positive correlation ($R = 0.196$, *p-value* = 0.049, 95%CI).

The diagnostic performance of GPC-3 and AFP gene in the HCC group is summarized in Table 5. The accuracy of GPC-3 gene expression for HCC diagnosis was estimated by the receiver operating characteristic (ROC) curve. The result showed that the area under the curve (AUC) for liver GPC-3 gene was 0.850 at a cut-off of -1.34, while the AUC for plasma GPC-3 gene was 0.867 at a cut-off of -10.8 (Figure 3 A–B).

Table 5 Diagnostic performance of liver GPC-3 gene, plasma GPC-3 gene, liver AFP gene and plasma AFP gene

	Liver GPC-3 mRNA	Plasma GPC-3 mRNA	Liver AFP mRNA	Plasma AFP mRNA
Sensitivity (%) (95% CI)	100	83	60	16
Specificity (%) (95% CI)	66.6	90	22.22	88
Positive predictive value (%) (95% CI)	66.6	83.3	30	50
Negative predictive value (%) (95% CI)	100	90	50	61.5
Diagnostic accuracy (%) (95% CI)	80	87.7	35.71	60

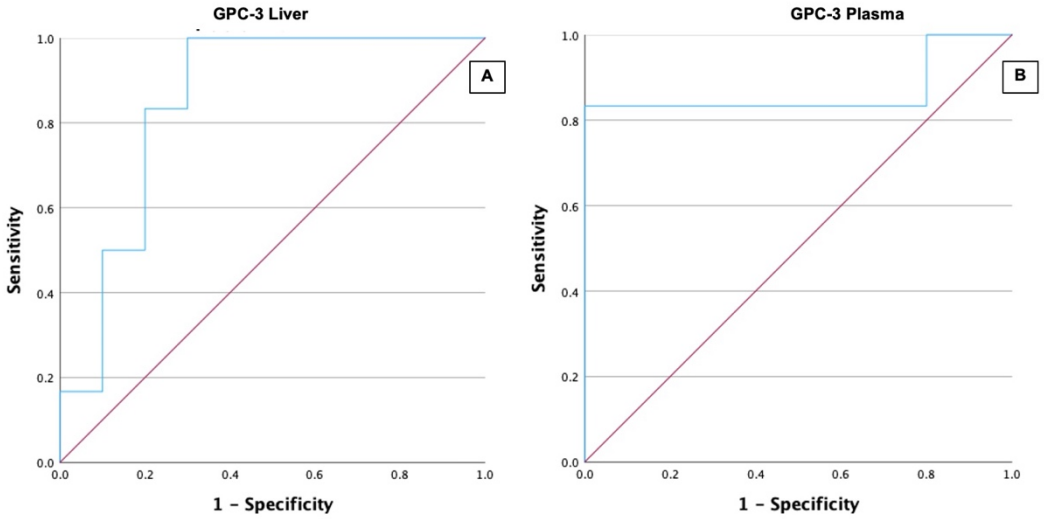

Figure 3 The ROC curve of GPC-3 mRNA in HCC group. (A) ROC curve of liver GPC-3 mRNA (AUC=0.850, cut off -1.34). (B) ROC curve of plasma GPC-3 mRNA (AUC=0.867, cut off -10.8).

DISCUSSION

This study demonstrated the expression patterns of AFP and GPC-3 mRNA in HCC, and non-HCC dogs. The results showed that dogs with HCC had a low positive rate of AFP gene expression in their liver tissue, while plasma samples showed a higher detection rate of AFP mRNA. In contrast to the previous research, the AFP gene expression levels in the liver samples from the HCC group seemed lower than those from the non-HCC groups. In dogs, AFP gene expression is recognized as a valuable marker for HCC detection. However, the difference in AFP gene expression levels among individuals with similar conditions affects the establishment of definitive diagnostic or prognostic thresholds (Wang et al., 2007). Other liver diseases, such as chronic hepatitis or cirrhosis, may also elevate AFP gene expression levels. Furthermore, Li et al. (2013) revealed that HCC can be negative for AFP gene expression. Therefore, AFP gene detection may not be sufficiently sensitive or specific to capture all cases of HCC (Li et al., 2013).

According to the present study, all samples in each group expressed GPC-3 gene. The GPC-3 gene expression in liver samples of HCC and non-HCC groups was no significant difference. Furthermore, the GPC-3 gene expression in plasma tended to be higher in HCC groups. To the best of our knowledge, the study of GPC-3 gene expression in the veterinary field remains limited. The present study, therefore, is the first to identify this gene in dogs. In human medicine, several studies have described the detection of GPC-3 gene in HCC patients, whereas healthy individuals did not express this gene (Nakatsura et al., 2003; Abdelgawad et al., 2013). Therefore, GPC-3 gene has been used as a diagnostic marker for patients with liver cancer.

Moreover, in our study, the GPC-3 gene expression level was observed in non-HCC dogs. The GPC-3 gene expression has also been detected in normal liver tissues, hepatitis, hepatic regeneration, or degenerative hepatopathy in humans (Baumhoer et al., 2008; Wu et al., 2016). Therefore, the assessment of GPC-3 gene expression alone may not be adequate for diagnosing HCC in dogs.

The present study also found a notable correlation in GPC-3 gene expression levels between liver tissue and plasma samples of the HCC group. Bao-ding Li (2006) reported a positive correlation between tissue and peripheral GPC-3 gene expression levels (Li et al., 2006). The association between liver GPC-3 and plasma

GPC-3 gene expression levels indicates that changes in GPC-3 gene expression levels in the liver are reflected in the plasma. This relationship can be used to detect and monitor HCC non-invasively. By measuring the GPC-3 gene expression levels in plasma, veterinarians can potentially identify HCC earlier and track the effectiveness of treatment, which is advantageous since it avoids the need for more invasive procedures like liver biopsies. This kind of correlation is important for improving diagnostic and treatment outcomes in dogs.

Furthermore, the HCC group demonstrated that the levels of liver tissue and plasma GPC-3 gene expression were related to AST levels. Unfortunately, there have been no studies reporting the relationship between GPC-3 gene expression and AST levels. We postulate that the relationship between AST levels and GPC-3 gene expression in dogs with HCC may reflect the severity of the disease and the rate of hepatic cell mortality.

This study also demonstrated the diagnostic performance of both GPC-3 and AFP gene expression for identifying canine HCC. The results showed that GPC-3 gene expression exhibited significantly more sensitivity than AFP gene expression (sensitivity values of 100% for liver samples and 83% for plasma samples). This suggests that GPC-3 gene is a good biomarker for detecting canine HCC development. Moreover, in this study, the specificity of GPC-3 gene expression was 66.6% for liver samples and 90% for plasma samples, indicating that although GPC-3 gene expression can accurately identify a high proportion of HCC cases, a number of false positives emerged, especially in the sample from liver tissues. Several factors affect specificity values, including the sample types used, the populations tested, and the cut-off values for defining positivity (Yao et al., 2013; Wang et al., 2014; Jing et al., 2017). Although the combination of high sensitivity and moderate specificity of GPC-3 gene expression can investigate HCC development, other liver diseases or physiological states might cause excessive levels of GPC-3 gene expression. Therefore, the detection of GPC-3 gene expression levels should be analyzed together with clinical findings and other diagnostic tests to ensure accurate diagnosis.

In the present study, HCC is found in female dogs, in contrast to a previous study that described HCC prevalence in male dogs (Leela-Arporn et al., 2019a). In human medicine, one study described that HCC cells from men and women expressed sex hormone receptors, especially in males (Gibson et al., 2022). Furthermore, several studies demonstrated that men were more likely to develop HCC than women (London, 2011; Gibson et al., 2022; McGlynn and Toh et al., 2023). However, the influence of sex hormones on the development of canine HCC is still unclear. Therefore, further study should be conducted to investigate the levels of sex hormone receptor expression in canine HCC.

This study found a variety of clinical signs in dogs with HCC. Inappetence, abdominal distension, and abdominal pain were the most common symptoms. However, these signs were considered non-specific (Liptak et al., 2004a). For concurrent diseases, hyperadrenocorticism was associated with HCC development. In our study, only 50% of the dogs with HCC had evidence of hyperadrenocorticism. This disease interfered with and stimulated the metabolism via prolonged exposure to the cortisol hormone, resulting in hepatocyte mutation and con HCC development (Leela-Arporn et al., 2019a; Leela-Arporn et al., 2019b).

The biochemical profiles of this study revealed that ALP levels more than three times the upper reference limit were illustrated in most HCC cases, whereas ALT levels of more than three times the upper reference limit were found in 50% of HCC cases. In contrast, ALP and ALT levels of more than three times the upper limit were found in 83.3% and 32.3% of non-HCC cases, respectively. Elevated AST was also observed in the HCC group, indicating hepatocellular injury or death, reflecting the aggressive progression of HCC (Leela-Arporn et al., 2019a; Leela-Arporn et al., 2019b).

CONCLUSIONS

This preliminary report reveals that GPC-3 gene expression can be detected in HCC dogs and may be used to distinguish HCC from other diseases. The clinical implications of GPC-3 gene expression and clinicopathological characteristics are essential parameters for diagnosis, treatment decisions, treatment responsiveness, and prognosis in canine HCC.

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AUTHOR CONTRIBUTIONS

Aphinan Phosri, Kidsadagon Pringproa, Niyada Thitaram, Pinkarn Chantawong and Phongsakorn Chuammitri, Atigan Thongtharb contributed to the conception and design of the study. Aphinan Phosri and Atigan Thongtharb performed material preparation, data collection, and analysis. Phongsakorn Chuammitri supervised the laboratory study. Aphinan Phosri wrote the first draft of the manuscript, and all authors contributed to reviewing and editing the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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