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Evaluation of serum alpha-fetoprotein in dogs with hepatic tumors: A diagnostic study

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Abstract

The present study was conducted to evaluate the diagnostic utility of serum alpha-fetoprotein (AFP) as a non-invasive biomarker in dogs with hepatic tumors. Dogs presented with clinical signs suggestive of hepatic disease were subjected to ultrasonographic examination, and those showing hepatic masses or altered echotexture were considered suggestive of hepatic tumors. Further it was confirmed by fine needle aspiration cytology, and only twelve confirmed cases were included in the study for the estimation of serum alpha foetoprotein. Blood was collected from these dogs for estimation of AFP levels using the iChroma™ fluorescence immunoassay method. Serum samples from apparently healthy dogs were also analyzed for comparison. The results revealed that all confirmed hepatic tumors cases exhibited markedly elevated AFP levels exceeding 500 ng/mL, whereas healthy dogs showed AFP concentrations within the range of 60–80 ng/mL. The clear difference between normal and affected animals indicates a strong association between elevated serum AFP levels and hepatic tumor. The study highlights that serum AFP estimation using a rapid immunoassay system can serve as a reliable, minimally invasive diagnostic tool and may be effectively used as an adjunct in the diagnosis of hepatic tumor in dogs.

Keywords: Hepatic tumor, alpha foetoprotein, i chroma, ultrasonography, cytology

Introduction

Alpha-fetoprotein (AFP) is a fetal serum protein predominantly produced by the liver and yolk sac during embryonic development. In healthy adult animals, its concentration remains extremely low or undetectable. However, under pathological conditions, particularly hepatic neoplasia, AFP production may be reinitiated due to alterations in hepatocellular differentiation. This re-expression is associated with the return of fetal gene expression patterns in malignant hepatocytes (Abelev & Eraiser, 1999)^[1]. Elevated serum AFP concentrations have been reported in conditions involving active hepatocellular proliferation and regeneration. In dogs, increased AFP levels are most commonly associated with hepatocellular carcinoma, while moderate elevations may occasionally be observed in other hepatic disorders or non-hepatocellular tumors (Yamada *et al.*, 1999)^[8]. The underlying mechanism is believed to involve cellular dedifferentiation, where transformed hepatocytes regain the ability to synthesize proteins typically restricted to fetal life (Johnson *et al.*, 1995)^[3]. Previous studies have demonstrated that AFP serves as a useful biomarker for hepatic tumors, particularly in distinguishing neoplastic from non-neoplastic liver conditions. Markedly elevated AFP concentrations are more frequently seen in hepatocellular carcinoma and, to a lesser extent, in cholangiocellular carcinoma, whereas minimal changes are usually observed in benign hepatic diseases or tumors of stromal origin (Mizejewski, 2001)^[6]. Despite its limitations as a standalone diagnostic marker, AFP measurement provides valuable supportive information when interpreted alongside imaging techniques such as ultrasonography and confirmatory cytological or histopathological findings (Stokol *et al.*, 2000)^[7]. In recent years, there has been growing interest in incorporating serum biomarkers into routine diagnostic protocols to improve the early detection and characterization of hepatic diseases in dogs. In this context, the present study was undertaken to evaluate the diagnostic utility of serum alpha-

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fetoprotein levels in dogs with hepatic tumors and to assess its effectiveness as a minimally invasive adjunct tool in clinical practice.

Materials and Methods

Dogs presented to the Small Animal Medicine Outpatient Unit of Madras Veterinary College with clinical signs such as abdominal distension, icterus, cachexia, and edema were referred for ultrasonographic examination of the abdomen. A detailed ultrasonographic evaluation was performed in all cases, and dogs showing hepatic abnormalities like focal or multifocal masses, altered echotexture, irregular margins, or nodular lesions suggestive of liver tumors were selected for the study (Fig.1-4). These suspected cases were further subjected to ultrasound-guided fine needle aspiration cytology under aseptic conditions. Smears were prepared, stained using Giemsa stain, and examined microscopically under oil immersion, and only those cases confirmed as hepatic tumor were included in the study (Fig.5-6). Twelve tumor confirmed dogs were taken for the study. Approximately 2–3 mL of blood was collected aseptically from the cephalic or saphenous vein from the affected dogs. Six apparently healthy dogs presented for the vaccination or general checkup were randomly selected and collected the blood to obtain normal reference values for comparison of parameters under study. The samples were allowed to clot and then centrifuged to separate serum. Serum alpha-fetoprotein levels were estimated using the ichroma™ fluorescence immunoassay kit based on antigen–antibody reaction principles, the more antigen in sample forms the more antigen-antibody complex and leads to stronger intensity of fluorescence signal on detector antibody, which is processed by instrument for ichroma™ tests to show AFP concentration in sample and the procedure was carried out according to the manufacturer's instructions.

Results and Discussion

In the present study, apparently healthy dogs showed AFP levels within the range of 60–80 ng/mL. Out of twelve, nine dogs exhibited markedly elevated serum alpha-fetoprotein (AFP) concentrations, with values exceeding 500 ng/mL, whereas the other three dogs exhibited the value around 300–500 ng/mL. A serum AFP level of 250 ng/mL, determined by enzymetry, was established as the threshold to differentiate between hepatic neoplasia and non-neoplastic liver conditions (Lowseth *et al.*, 1991)^[5]. The findings of the present study are in agreement with those of Yamada *et al* (1999)^[8] who reported that AFP levels in healthy dogs were typically below 70 ng/mL, while dogs with hepatocellular carcinoma showed markedly elevated values, often exceeding 1400 ng/mL. Serum AFP concentrations in dogs with hepatic tumors other than hepatocellular carcinoma and cholangiocellular carcinoma were not readily differentiated from those seen in dogs with non-neoplastic liver conditions. This observation is anticipated, as these tumor types arise from stromal tissues rather than the hepatic parenchyma. Because AFP synthesis is mainly carried out by hepatocytes, neoplasms originating from stromal elements are not expected to secrete appreciable levels of AFP (Lowseth *et al.*, 1991)^[5]. The consistent elevation of AFP in almost all affected cases indicates a strong association between increased serum AFP levels and hepatic tumor in dogs. However, it is important to note that the iChroma™ fluorescence immunoassay kit used in this study has an upper detection limit of 500 ng/mL, and values above this threshold are reported only as >500 ng/mL without

providing exact quantitative measurements. This limitation prevented precise determination of the actual AFP concentrations in cases with markedly elevated levels, which may have underestimated the extent of increase in different kinds of hepatic tumors of dogs. The uniformly elevated AFP levels observed in the present study, despite the upper detection limitation of the assay, further emphasize its diagnostic significance. Additionally, AFP has been reported to correlate with tumor burden and may decrease following treatment, indicating its potential role in disease monitoring (Kitao *et al.*, 2006)^[4].



Fig 1: Predominantly hyperechoic heterogeneous mass seen in the left lateral lobe of liver parenchyma.



Fig 2: Solitary circumscribed isoechoic mass seen in the right medial lobe of liver parenchyma



Fig 3: Heterogenous huge mass with irregular borders occupying the entire lobes of liver parenchyma



Fig 4: Multiple small hypoechoic nodules seen in entire lobes of liver parenchyma

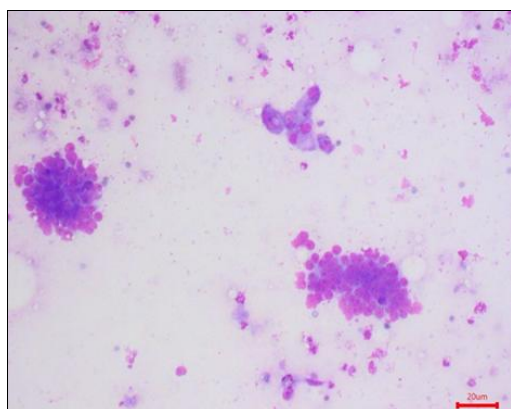


Fig 5: Cellular crowding, overlapping and loss of normal architecture was observed.

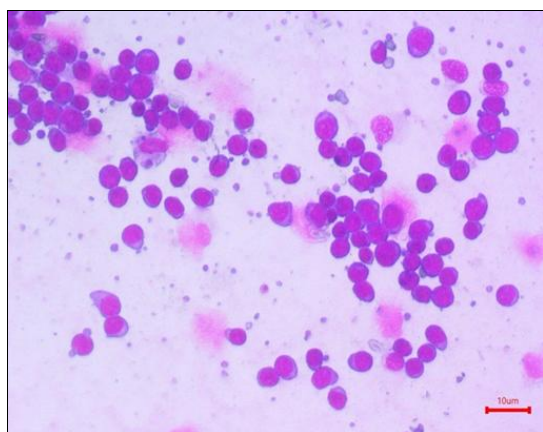


Fig 6: Numerous round to polygonal cells arranged in clusters and occasionally acinar like groupings. Cells exhibit moderate to marked anisocytosis and anisokaryosis, with high nuclear to cytoplasmic ratio.

Conclusion

The present study concludes use of the iChroma™ fluorescence immunoassay method provided a rapid and practical approach for AFP estimation in a clinical setting. Despite its limitation in quantifying values above 500 ng/mL, the clear differentiation between normal and affected dogs suggests that AFP estimation remains a valuable, minimally invasive adjunct diagnostic tool for hepatic tumors in dogs. However, AFP should always be interpreted in conjunction with clinical findings, ultrasonography, and cytological or histopathological confirmation for accurate diagnosis.

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Conflict of Interest

The authors declare no conflict of interests

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