

PLASMA TAURINE ESTIMATION IN DILATED CARDIOMYOPATHY OF LABRADOR RETRIEVERS

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ABSTRACT

The objective of this study was to quantify the concentrations of taurine in the plasma of Labrador retrievers with clinical disease of Dilated Cardiomyopathy (DCM) and to compare with the healthy Labrador retrievers. The study was conducted in thirty clinical cases of Dilated cardiomyopathy (DCM) in Labrador Retrievers and in thirty healthy Labradors. The plasma taurine estimation by HPLC showed a highly significant decrease in taurine levels (18.712 ± 3.53 nmol/mL) in dogs with Dilated cardiomyopathy compared to healthy dogs (51.022 ± 5.55 nmol/mL). Therefore, it was concluded that there was a significant role of plasma taurine in the pathogenesis of Dilated cardiomyopathy in Labrador Retrievers. This pilot study justifies the supplementation of taurine in DCM of Labradors and also it opened up a new area for extensive research at a larger scale involving multi centres. In addition a commercially viable method for estimation of taurine was developed.

INTRODUCTION

Idiopathic, Dilated Cardiomyopathy (DCM) is a condition of unknown aetiology, characterized by progressive dilatation of one or both ventricles with severe impairment of systolic function in the absence of congenital, coronary arterial, hypertensive, vascular, pulmonary parenchymal, valvular, or other cardiovascular disorders (Cobb 1992). Although it is a disease of uncertain aetiology, various factors such as genetic and nutritional were proposed. Among

nutritional, low L-carnitine in certain Boxers and low taurine in Cocker Spaniels and Newfouland were reported. Therefore, it is possible that Labrador Retrievers affected with DCM may have low taurine levels as this breed had originated from Newfouland.

Kramer *et al.* (1995) in a study of 76 dogs with DCM reported that plasma taurine concentration was low (< 25 nmol/mL) in 17% (13/76) of the dogs. Seven of the 13 dogs with low plasma taurine

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concentrations were Cocker Spaniels and Golden Retrievers. Belanger *et al.* (2005) reported taurine deficiency in a family of five Golden Retrievers. Backus *et al.* (2006) in a study of 216 Newfoundland a giant dog breed with high incidence of idiopathic DCM (1.3-2.5%), reported low plasma taurine in 8% of dogs. Of the nine taurine-deficient, clinically evaluated dogs, three had DCM that was reversed by taurine supplementation.

Taurine, an amino acid that exhibits anti-angiotensin II and osmoregulatory activity, is found in very high concentration in the heart. When the intracellular content of taurine is dramatically reduced, the heart develops contractile defects and undergoes an eccentric form of hypertrophy. The development of myocyte hypertrophy has been largely attributed to angiotensin II, whose growth properties are antagonized by taurine. Overt heart failure is usually associated with myocyte death, including death due to angiotensin II-induced apoptosis (Schaffer *et al.* 2003).

The proposed research will estimate the prevalence of a possible unrecognized, widespread, taurine deficiency in the Labrador retriever breed leading to Dilated cardiomyopathy. The objective of the project is to quantify the concentrations of taurine in the plasma of Labrador retrievers with clinical disease of Dilated Cardiomyopathy (DCM) and to compare with the healthy Labrador Retrievers.

MATERIALS AND METHODS

The clinical cases of Dilated Cardiomyopathy was identified by the clinical findings, radiography, electrocardiography

and confirmed with echocardiography. Laboratory investigations were done to rule out other coexisting diseases. The blood samples from the confirmed cases of Dilated Cardiomyopathy were collected in a heparinised polyurethane tubes and the plasma separated by centrifugation and stored at -20°C for estimation of taurine. The control group includes healthy Labrador Retrievers attending hospital for regular health check up and vaccination. Plasma samples from healthy Labradors were collected and submitted for taurine estimation.

GROUPS OF CLINICAL STUDY

Group I: Thirty apparently healthy Labrador Retrievers acting as Control group

Group II: Thirty confirmed cases (twenty males and ten females) of Labrador Retrievers with Dilated

Cardiomyopathy

Physical examination was carried out as suggested by McCurin and Poffenbarger (1991). The animals under study were subjected to thoracic radiography to record the changes and Vertebral Heart Score (Plate-1) was calculated as suggested by Buchanan and Bucheler (1995). Electrocardiography was recorded as per the standard procedure described by Tilley and Smith (1997) using Welch Allyn ECG monitor. Echocardiographic examinations were performed as suggested by Boon (1998) using ALOKA SSD 3500 ultra sound system with a Phased array Transducer of 3.0 – 6.0 MHz to obtain Two dimensional, M- mode, Pulsed wave and color flow Doppler echocardiography images of heart.

The following guidelines for the diagnosis of DCM as proposed by McEwan *et al.* (2003) were used with little modification for clinical cases of Dilated cardiomyopathy to be included in the study group:

1. Left ventricular dilation (especially in systole but also in diastole).
2. Depressed systolic function.
3. Altered geometry of the left ventricle (increased sphericity).
4. Left or bi-atrial enlargement
5. M-mode fractional shortening of < 25%.
6. Left ventricular ejection fraction less than 40%.
7. Increased mitral valve M-mode E point to septal separation (EPSS).
8. No co-existing diseases

Procedure of Plasma Taurine estimation by High Performance Liquid Chromatography (HPLC)

The mobile phase composition of HPLC consists of 0.02M phosphate buffer and the acetonitrile in the ratio of 84:16 and the PH 6 was maintained using 0.1% acetic acid. C18 column of 250 X 4.6mm and particle size of 5 μ was used in this method. The temperature was set at 30° C throughout the analysis. RF fluorescence detector was used at the excitation wavelength of 350nm and emission wavelength of 570nm. The

injection volume of 10ul is injected in to the HPLC system. The flow rate was maintained at 1.2ml/minute.

Preparation of Standard Solutions

Standard taurine solutions were made as close to 10, 20 and 50 ppm as possible. A solid taurine sample (Sigma-Aldrich) of 0.2519 g was dissolved in DI water in a 500-mL volumetric flask and diluted to the mark. Three 50-mL volumetric flasks were obtained and 1.0, 2.0 and 5.0 mL of the previous solution were added to each, respectively. The flasks were then diluted to volume with DI water. The intra- and the inter-day coefficients of variation for the method were 5.3% and 7.7%, respectively. The calibration curve was linear from 0.1 μ mol/L to 30.0 μ mol/L with a correlation coefficient of 0.9995. A results summary for each of the three standards. Sample Conc (ppm) Run 1 Area (mAU·s) Run 2 Area (mAU·s) Run 3 Area (mAU·s) Run 4 Area (mAU·s) Standard 1 9.968 54.5 54.3 51.3 52.4 Standard 2 19.94 113.4 114.1 125.7 125.9 Standard 3 49.84 307.0 306.9 339.9 339.1. LOD 50 μ g/Kg, LOQ 75 μ g/Kg. Recovery percentage 85-91%. Linearity range The intra- and the inter-day coefficients of variation for the method were 5.3% and 7.7%, respectively. The calibration curve was linear from 0.1 μ mol/L to 30.0 μ mol/L with a correlation coefficient of 0.9995.

RESULTS AND DISCUSSION

The average age of affected dogs was 6.68 \pm 0.47 years, and the incidence in male dogs were 67 per cent (20/30) and female dogs were 33 per cent (10/30). The physical examination findings recorded

were dyspnoea in 100 per cent (30/30) of dogs, ascites in 97 per cent (29/30) of dogs, gallop rhythm in 87 per cent (26/30) of dogs, systolic murmur in 73 per cent (22/30) of dogs, limb oedema in 53 per cent (16/30) of dogs and weak femoral pulse in 50 per cent (15/30) of dogs. In ECG, normal sinus rhythm was appreciated in 63 per cent (19/30) of dogs and ST depression in 70 per cent (21/30) of dogs that were affected with DCM. The abnormal rhythm includes atrial fibrillation in (fig 1) 27 per cent (8/30) of dogs and ventricular premature contraction in 10 per cent (3/30) of dogs.

In radiography, (fig 2) highly significant ($p \leq 0.01$) increase in the vertebral heart score was noticed in DCM (12.51 ± 0.14) dogs compared to control (10.78 ± 0.03). The other major radiographic abnormalities in the DCM affected dogs were cardiomegaly in 100 per cent (30/30) of dogs, pulmonary edema in 80 per cent (24/30) of dogs and pleural effusion in 20 per cent (6/30) of dogs.

In 2-D echocardiography, highly significant ($p \leq 0.01$) increase in the LA (4.09 ± 0.05 cm), Ao (2.08 ± 0.05 cm), LA/Ao (2.01 ± 0.06), EDV (95.16 ± 1.97 ml) and ESV (67.30 ± 2.36 ml) were noticed in DCM dogs compared to control. Highly significant decrease in the EF (29.11 ± 2.13 per cent) was noticed in DCM dogs compared to control. In M-Mode, highly significant increase in the LVIDd (5.84 ± 0.13 cm), LVIDs (5.05 ± 0.12 cm), RVIDs (1.24 ± 0.09 cm) and EPSS (1.92 ± 0.09 cm) were noticed in DCM dogs compared to control whereas highly significant decrease in the IVSs (0.81 ± 0.04 cm), LVPWs (0.97 ± 0.03 cm), FS (13.45 ± 0.73 per cent) and IVS FT

(15.99 ± 2.38 per cent) were noticed in DCM dogs compared to control (fig 3).

The present physical findings, electrocardiography, radiography and echocardiography findings in dilated cardiomyopathy affected dogs are consistent with the findings of previous reports by Calvert *et al.* (1982); Atkins and Snyder (1992); Monnet *et al.* (1995); Tidholm and Jonsson (1996); McEwan *et al.* (2003) and Borgarelli *et al.* (2006)

The plasma taurine estimation by HPLC showed a highly significant decrease in taurine levels (18.712 ± 3.53 nmol/mL) in dogs with Dilated cardiomyopathy compared to healthy dogs (51.022 ± 5.55 nmol/mL). Delaney *et al.* (2003) reported plasma taurine levels of less than 40 nmol/mL as critically low. In the present study the mean plasma taurine levels in DCM dogs were well below the critical levels reported Delaney *et al.* 2003. When compared, no significant changes in ECG, radiograph, echocardiography and plasma taurine levels were recorded in between the males and female Labrador retrievers with Dilated Cardiomyopathy.

Schaffer *et al.* (2003) reported a very high concentration of taurine in the heart and exhibits anti-angiotensin II and osmoregulatory activity. The authors proposed that when the intracellular content of taurine is dramatically reduced, the heart develops contractile defects and undergoes an eccentric form of hypertrophy. The development of myocyte hypertrophy has been largely attributed to angiotensin II, whose growth properties are antagonized by taurine. Overt heart failure is usually

associated with myocyte death, including death due to angiotensin II-induced apoptosis.

CONCLUSION

From the study, it was concluded that there was a significant ($p < 0.01$) decrease in plasma taurine levels in DCM Labrador retrievers compared to normal Labradors and therefore, taurine deficiency has a significant role in the pathogenesis of DCM in Labrador retriever. This pilot study justifies the supplementation of taurine in DCM of Labradors and also it opened up a new area for extensive research at a larger scale involving multi centres. In addition a commercially viable method for estimation of taurine was developed.

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ACKNOWLEDGEMENT

The authors thank Tamil Nadu Veterinary and Animal Sciences University for providing their corpus funds to carry out this research work.

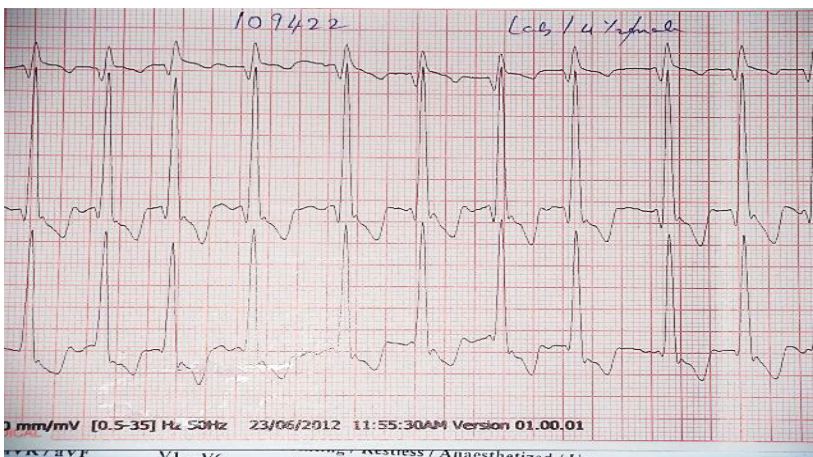
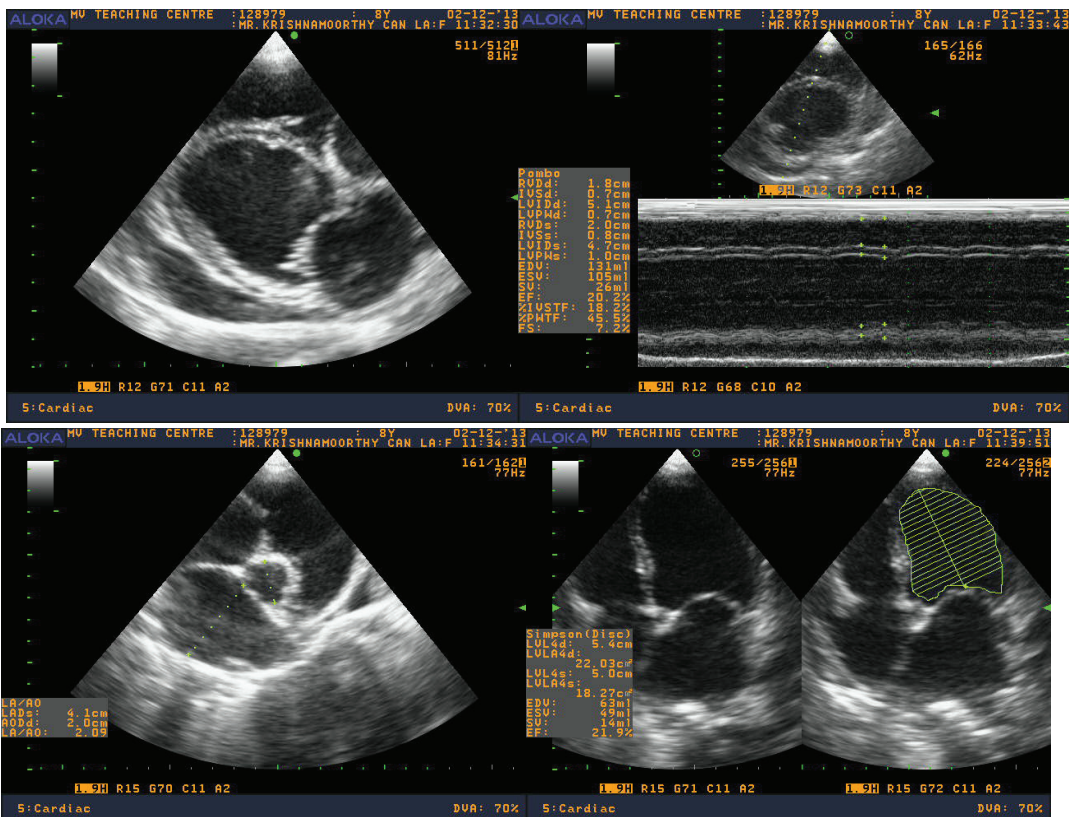


Figure 1 – Electrocardiographic findings in DCM-Atrial Fibrillation

Figure 2- cardiomegaly with pulmonary edema



Figure 3 – Echocardiographic findings in DCM



Echocardiograph showing dilated heart chambers and reduced contractility