Comparative assessment of N-terminal pro-B-type natriuretic peptide, nitric oxide and echocardiography for diagnosis of dilated cardiomyopathy in dogs

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Summary

The aim of the study was to evaluate the clinical usefulness of N-terminal pro-brain natriuretic peptide (NT-Pro BNP) and nitric oxide (NO) with echocardiography (gold standard test) in diagnosis of canine cardiomyopathy. Out of 374 cases, 82 cases showing cardiovascular abnormalities including 16 cases of cardiomyopathy were further selected for standardization of biomarkers. Significant (P<0.01) increase in plasma NT-Pro BNP was found in dogs with cardiomyopathies. The plasma concentration of NO showed non-significant increase in comparison to healthy dogs. The sensitivity of NT-Pro BNP and NO was 72.20% and 62.50%, with specificity 93.90% and 89.40%, respectively when compared with echocardiography. The correlation between echocardiography and NT-Pro BNP was 89.30% with kappa value of 0.675 suggesting substantial agreement between the two tests. The positive and negative predictive value was estimated at 0.765 and 0.925, respectively. It is concluded that NT-Pro BNP and NO may be an alternate diagnostic tool for diagnosis of cardiomyopathy where the facility of echocardiography is not available.

Key words: Cardiomyopathy, Echocardiography, N-terminal pro-brain natriuretic peptide, Nitric oxide

Introduction

Cardiac diseases are a potential cause of death in canine besides prolonged suffering and physiological disability (Egenvall et al., 2006). The estimated morbidity related to canine cardiac diseases has been found to be 1 in every 10 dogs (Dove, 2001). Besides mortality, cardiovascular diseases induce animal's suffering and worsening quality of life. Cardiomyopathy represents a group of heterogeneous conditions that affect the structure and functions of the heart and is characterized by dilation or hypertrophy of all the cardiac chambers, in particular the left ventricle (Tidholm and Jonsson, 2005). Cardiomyopathy is the most prevalent acquired cardiac disease in canine (Baumgartner and Glaus, 2004) and may be idiopathic (dilated, restrictive, arrhythmogenic right ventricular) or specific (hypertensive, endocrine, metabolic and ischemic) in origin (Israël, 2003). Dilated cardiomyopathy (DCM) condition impairs the contractility of myocardium leading to systolic dysfunctions; however, dysfunctions have also been reported diastolic (Richardson et al., 1996; Tidholm and Jonsson, 2005).

The routine techniques clinically adopted in assessing cardiac function are indirect serum biochemical markers, electrocardiography (ECG), radiography, and echocardiography (Sahn *et al.*, 1978). Electrocardiography and radiography are less sensitive and specific and could detect the disease at an advanced stage. In India, facilities and expertise of echocardiography are yet to be available in all veterinary hospitals. In the last decade, cardiac biomarkers, primarily cardiac troponin and natriuretic peptides, have become a mainstay for both the diagnosis, and therapeutic prognosis of canine cardiomyopathy (Schober *et al.*, 1999; Boswood *et al.*, 2008).

The chronic overstimulation of the renin-angiotensinaldosterone system occurs with heart disease which leads to cardiac enlargement, volume overload, and congestive heart failure. The natriuretic peptides counteract this activity by stimulating natriuresis, renal blood flow, diuresis, and vasodilation as well as enhancing diastolic heart function (Boswood et al., 2008). Circulating atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) levels are increased, primarily in response to increased myocardial wall stress (Boswood et al., 2008). The increased concentrations of ANP and BNP were linked to various causes including myocardial infarction, cardiac hypertrophy and heart failure (Morita et al., 1993; Nishikimi et al., 1996; Koglin et al., 2001). NT-Pro BNP has been proved to be a useful marker for diagnosis of heart disease in human medicine for more

than a decade (Morita *et al.*, 1993; Koglin *et al.*, 2001). Canine proBNP and human proBNP are only 46% homologous compared to 87% homology for canine and human proANP and it is necessary to use canine specific diagnostic markers to evaluate proBNP levels (Seilhamer *et al.*, 1989; Biondo *et al.*, 2002).

Recent studies on canine NT-Pro BNP assay have been found useful in assessment of heart disease in dogs (DeFrancesco *et al.*, 2007; Boswood *et al.*, 2008; Piantedosi *et al.*, 2009) either alone or in conjunction with physical examination, thoracic radiography, and ECG (DeFrancesco *et al.*, 2007; Sastravaha *et al.*, 2010).

Nitric oxide (NO) is a member of a family of labile biological mediators termed gasotransmitters (Szabo, 2007). Nitric oxide is generated in mammals, including humans, by nitric oxide synthases (NOS) and plays a prominent role in controlling blood pressure via the regulation of vascular tone (Ignarro, 1999). Pathophysiological roles of NO are reported in various disorders of the heart (Kelly et al., 1996). Patients with cardiovascular disease showed significantly higher concentration of NO in comparison to healthy patients and hence can be regarded as emerging markers of oxidative stress mediated myocardial damage (Das et al., 2010). The measurement of plasma NO has also been shown to be useful in predicting congenital cardiac diseases (Takaya et al., 2000). In the last decade, NT-Pro BNP and NO have been used for evaluating cardiovascular diseases in human. However, reports on the clinical usefulness of these plasma markers for diagnosis of canine cardiomyopathy in India are still to be validated. Therefore, the present study was conducted to evaluate the diagnostic capabilities of NT-Pro BNP and NO in diagnosis of canine cardiomyopathy.

Materials and Methods

The study was conducted on clinical cases of dogs reported during the year (2010-2012) at the Referral Veterinary Polyclinic, Indian Veterinary Research Institute, Izatnagar, India after approval of the institute ethics committee. Eighty-two dogs were selected after screening of 374 clinical cases showing signs suggestive of cardiovascular abnormalities. Six healthy dogs maintained in the Division of Animal Nutrition, IVRI, Izatnagar, were selected as control for standardization of biomarkers.

The ECG was recorded in standard body position (Tilley, 1985) using standard ECG recorder (6 leads), Cardiart-408-BPL (service power supply Class 1, 220-240 V \pm 10%; 50-60 Hz) at paper speed of 50 mm sec with sensitivity of (1 cm=1 mV) without use of filter. A minimum of 10 complexes in bipolar limb leads (I, II, III) and augmented unipolar limb leads (aVR, aVL and aVF) were recorded. Echocardiographic examinations were performed using two-dimensional and motion mode (M-mode) echocardiography machine (Pie Medicals, Netherlands) with 5 MHz annular array transducer. Images were recorded with the help of thermo printer while measuring cardiac parameters (Mitsubishi, Japan).

Blood was collected from saphenous vein in a K3 EDTA coated vacutainer. Plasma was collected and stored at -20°C till analyzed. Plasma NT-Pro BNP concentration was measured using commercial sandwich enzyme immune assay (Uscn Life Science Inc., Wuhan, China) using the method of Oyama et al. (2008). Optical density (OD) of standard and samples were read at 450 nm by ELISA reader. By plotting standard curve on loglog graph paper, with NT-Pro BNP concentration on Xaxis and absorbance on Y-axis, concentration in the sample was determined by regression analysis and expressed as pg/ml. Nitric oxide concentration was measured in plasma following the methods described by Sastry et al. (2002). The absorbance of standards and samples were read at 545 nm against distilled water blank in ELISA reader. A multi-point linear standard curve of NO (derived from sodium nitrite, MW: 69, 99% purity, HiMedia, India), taking a range of concentrations from 0 to 200 µM was constructed and final values were obtained by plotting the absorbance in the curve and were expressed as µmolNO/mL plasma.

The data were analyzed statistically using standard methods (Snedecor and Cochran, 1994). The sensitivity, specificity and overall agreement, Kappa statistics (κ), along with the positive and the negative predictive values were analyzed as per the method suggested by Thrusfield (2006).

Results

The present study was conducted on 82 cases showing various cardiovascular abnormalities on ECG and 6 healthy dogs (control) for standardization of cardiac biomarkers NT-Pro BNP and NO. The significant (P<0.01) increase in plasma concentration of N-terminal pro-brain natriuretic peptide (NT-Pro BNP) was found in comparison to healthy dogs (5267.10 \pm 1149.38 and 783.26 ± 102.62 pg/ml), respectively. However, the plasma concentration of NO showed nonsignificant increase in dogs with various cardiovascular abnormalities in comparison to healthy $(22.97 \pm 5.57 \text{ and}$ $15.17 \pm 4.69 \ \mu mol/ml$), respectively. 16 out of 82 dogs showed evidence of cardiomyopathy in echocardiography. These 16 dogs with cardiomyopathy and 6 healthy dogs were further studied for comparison of cardiac biomarker (NT-Pro BNP and NO) between healthy and cardiomyopathic dogs. Significantly (P<0.01) higher concentration of NT-Pro BNP (7829.0 ± 1209.20 and 783.26 ± 102.63 pg/ml) was estimated in cardiomyopathy and healthy dogs, dogs with respectively. There was no significant change in plasma NO concentration between dogs with cardiomyopathy and healthy dogs (25.41 \pm 2.92 and 15.17 \pm 04.69 μ mol/L), respectively.

Comparison of these three tests revealed 16, 15 and 10 cases positive by echocardiography, NT-Pro BNP and NO, respectively. One case shown positive by echocardiography was negative by NT-Pro BNP whereas both the tests were in agreement in reporting 13 and 60 cases as positive and negative, respectively. Similarly 6 cases shown negative by NO were found positive by echocardiography whereas both echocardiography and NO had detected 10 and 59 cases as positive and negative for cardiomyopathy, respectively.

On further statistical analysis, the sensitivity of NT-Pro BNP and NO were 72.20% and 62.50%, with specificity 93.90% and 89.40% shown by both tests, respectively when compared with that of echocardiography as gold standard test. The overall agreement between echocardiography and NT-Pro BNP (89.30%) was higher than that of echocardiography and NO (Table 1).

Discussion

Echocardiography is a globally accepted diagnostic tool for evaluating cardiac chamber size, wall thickness, systolic and diastolic function of the heart. Two dimensional M-mode echocardiography has been traditionally used for cardiomyopathy and has been considered as gold standard (Schober *et al.*, 1999). However, owing to lack of technical expertise and instrumentation facility, many times it is not possible to perform it in all cases in India.

In the present study significant (P<0.01) increase in serum concentration of NT-Pro BNP was found in dogs positive for various cardiovascular abnormalities by ECG (n=82) in comparison with healthy dogs (n=6), respectively. Our findings are in agreement with the findings of Boswood et al. (2008) who reported a significant difference in concentration of NT-Pro BNP between dogs with heart disease, dogs with heart failure, and dogs with primary respiratory disease. Congestive heart failure had significantly higher NT-Pro BNP concentrations than those with respiratory disease. Median value was found as 2554 pmol/L and 357 pmol/L, respectively (Oyama et al., 2008). Similarly the concentration of NT-Pro BNP (7829.0 ± 1209.20 and 783.26 ± 102.63 pg/ml) has shown highly significant (P<0.01) increase in dogs diagnosed positive for cardiomyopathy by echocardiography (n=16) in comparison to healthy dogs (n=6), respectively. The results are in line with the finding of Noszczyk-Nowak (2011) who revealed that there was a significant increase in the level of NT-Pro BNP (median: 978 p mol/l) in the group of dogs with DCM. Similarly, Crivellente et al. (2011) suggest that NT-Pro BNP acts as an early

biomarker of cardiac changes, representing a sensitive and predictive marker of drug-induced cardiomyopathy.

In the present study, the sensitivity and specificity of NT-Pro BNP in dogs positive for cardiomyopathy when compared with echocardiography were 72.20% and 93.90%, respectively with overall agreement of 89.30% which was further supported by the kappa value of 0.675, suggesting a substantial agreement between the two tests. Our findings are in line with the findings of Oyama *et al.* (2007) who suggested that for diagnosis of occult case of cardiomyopathy, among NT-Pro ANP, BNP and troponins BNP had the best sensitivity and specificity, 95.2% and 61.9%, respectively.

In the present study, the cut-off value for NT-Pro BNP for diagnosis of cardiac myopathies was considered 783.26 ± 102.62 pg/ml (healthy dogs). The use of this cut off leads to positive predictive value of 76.5% (the probability for positive) and negative predictive value of 92.5% (the probability of absolute negative) with respect to echocardiography. This means that dogs with a positive test were 76.5% more likely to have cardiomyopathies, while dogs with a negative test were 92.5% more likely to not have cardiomyopathies. However, Boswood et al. (2008) suggests that a cut-off value of 210 pmol/L for NT-Pro BNP had a positive predictive value of 94% and a negative predictive value of 77% for predicting dogs with heart disease or heart failure. In another study, the serum NT-Pro BNP discriminated dogs with cardiomyopathy from healthy dogs with a positive predictive value of 97% and a negative predictive value of 61% when using a cut-off value of 445 pmol/L (Oyama et al., 2008). Oyama et al. (2009) reported the serum NT-Pro BNP cutoff concentration >1,158 pmol/L discriminated between dogs with congestive heart failure and dogs with primary respiratory tract disease with a sensitivity of 85.5% and a specificity of 81.3% while Ettinger et al. (2012) suggested that 900 pmol/L is the upper reference limit of plasma NT-Pro BNP concentration in dogs.

In the present study, the serum concentration of NO $(22.97 \pm 5.57 \text{ and } 15.16 \pm 4.69 \ \mu\text{mol/ml})$ showed nonsignificant increase in dogs positive for various cardiovascular abnormalities by ECG (n=82) in comparison to healthy dogs, respectively. Similarly, $(25.41 \pm 2.92 \text{ and } 15.17 \pm 04.69 \ \mu\text{mol/L})$ the concentration of NO has shown non-significant increase in dogs diagnosed positive for cardiomyopathy by

Table 1: Comparative evaluation of NT-Pro BNP and NO with that of echocardiography for detection of DCM in dogs

Tests	Gold standard echocardiography								
	Sensitivity	Specificity	Overall agreement	Positive predictive value	Negative predictive value	Kappa value	Positive likelihood ratio	Negative likelihood ratio	Diagnostic odds
NT-Pro BNP a=13, b=4, c=5, d=60	72.20%	93.90%	89.30%	0.765	0.925	0.675	11.917	0.296	40.300
Nitric oxide a=10, b=7, c=6, d=59	62.50%	89.40%	84.10%	0.588	0.908	0.507	5.893	0.419	14.048

a: Samples positive in both conventional and the gold standard tests, b: Samples positive in conventional but negative in the gold standard test, c: Samples negative in conventional but positive in the gold standard test, d: samples negative in both conventional and the gold standard. Kappa value >0.81: Almost perfect agreement, 0.61-0.80: Substantial agreement, 0.41-0.60: Moderate agreement, 0.21-0.40: Fair agreement, 0.01-0.20: Slight agreement, and 0.00: Poor agreement

echocardiography in comparison to healthy dogs, respectively. The finding of the present study is in contrast with the finding of Das *et al.* (2010) who reported significantly higher concentration of NO in patient with cardiovascular disease in human.

On further statistical analysis the sensitivity and specificity of NO when compared with echocardiography was 62.50% and 89.40% with overall agreement of 84.10 which was supported by kappa value of 0.507, suggesting a moderate agreement between the two tests. The findings of the present study are in line with the findings of Takaya *et al.* (2000) who suggested that measurement of plasma NO concentration can be useful in predicting cardiac disease.

From our study, it is concluded that plasma NT-Pro BNP shows promising results as a sensitive and specific marker for early diagnosis of canine cardiomyopathies which can be used as a reliable method of diagnosis where echocardiography is not clinically accessible. There was a moderate increase in the NO concentration in dogs with cardiomyopathies compared to healthy dogs, but NO can be an adjunct test to assess cardiac function as it has definite pathophysiological roles. Considering these facts, there is a need for further studies to ascertain its prognostic role as early biomarker in cardiomyopathic dogs.

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هدف از این تحقیق ارزیابی اهمیت بالینی ناتریوریک پپتید (NT-Pro BNP)، نیتریک اکساید (NO) و اکوکاردیوگرافی (تست تشخیصی طلایی) در تشخیص کاردیومیوپتی سگها میباشد. از 374 مورد ارجاعی، 82 بیمار مبتلا به اختلال قلبی عروقی تشخیص داده شد که 16 بیمار مبتلا به کاردیومیوپتی جهت ارزیابی و استاندارد کردن بیومارکرها انتخاب شدند. افزایش معنی داری (OS) و اکوکاردیوگرافی (تست MT-Pro BNP سگهای مبتلا به کاردیومیوپتی مشاهده گردید. غلظت پلاسمایی NO نسبت به سگهای سالم افزایش معنی داری (OS) و اکوکاردیوگرافی (تست MT-Pro BNP و NT-Pro BNP و ویژگی آنها به ترتیب 90/92% و 04/98% در مقایسه با اکوکاردیوگرافی تشخیص داده شد. همبستگی بین اکوکاردیورافی و 72/20% و ویژگی آنها به ترتیب 90/93% و 90/98% در مقایسه با اکوکاردیوگرافی تشخیص داده شد. همبستگی بین اکوکاردیورافی و 90 NT-Pro BNP، 90/00% با میزان کاپای 76/50 بود که نشان از هماهنگی زیاد بین این دو روش دارد. ارزش پیش گویانـه مثبت و منفی به ترتیب 1050 و 2000 تخمین زده شد. نتیجه گرفته میشود که احتمالاً میتوان از NT-Pro BNP و NT-Pro BNP

واژههای کلیدی: کاردیومیوپتی، اکوکاردیوگرافی، ناتریوریک پپتید N-terminal pro-B-type، نیتریک اکساید