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Evaluation of Cardiac Biomarkers for Early Diagnosis of Dilated Cardiomyopathy in Dogs

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Cardiac biomarkers play a crucial role in diagnosing heart disease in veterinary medicine. This collaborative study, involving 2497 dogs of various breeds, ages, and genders, was conducted collaboratively at the Department of Veterinary Clinical Medicine, College of Veterinary and Animal Science, RAJUVAS, Bikaner, and the Chandrika Chimanlal Doshi Cardiovascular Unit for Animals,

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Department of Veterinary Clinical Medicine, Ethics, and Jurisprudence, Bombay Veterinary College, Parel, Mumbai-12.Through meticulous examination, including historical analysis, clinical evaluation, and advanced diagnostic techniques like echocardiography, researchers conclusively diagnosed 29 cases of Dilated Cardiomyopathy (DCM).

Canine-specific cardiac Troponin I (cTnI) levels were measured, showing significant differences between healthy and DCM-affected dogs. Among DCM cases, bilaterally affected dogs exhibited the highest cTnI levels. Similarly, B-type natriuretic peptide (BNP) analysis revealed significant differences between healthy and DCM-affected dogs, with notably elevated levels in bilaterally affected dogs. N-Terminal-pro-BNP analysis also indicated significant differences between healthy and DCM-affected dogs.

However, atrial natriuretic peptide (ANP) analysis showed no significant differences between healthy and DCM-affected dogs, except for lower levels observed in right DCM cases. Endothelin 1 analysis showed non-significant differences between healthy and DCM-affected dogs, except for higher levels in bilateral DCM cases.

While these biomarkers offer promising diagnostic insights, they have limitations. Proper sample handling, longer turnaround times, and the necessity for concurrent analysis of renal and hepatic function are crucial considerations. Additionally, these biomarkers complement but do not replace other diagnostic methods such as radiography and echocardiography. Further research in larger dog populations is warranted to validate and refine these findings.

Keywords: Cardiac biomarker; cardiomyopathy; cardiac troponin I; endothelin-1.

1. INTRODUCTION

Cardiac biomarkers play a pivotal role in the diagnosis and management of Dilated Cardiomyopathy (DCM) in dogs. These biomarkers offer valuable insights into the structural and functional changes occurring in the heart, aiding in early detection, prognosis assessment, and treatment monitoring. Conventional diagnostic assays, including biochemical, hematological, and coagulation profiles, as well as urine and fecal analyses, lack the requisite specificity for detecting cardiovascular abnormalities despite potential alterations. Currently, diagnosis of occult cardiomyopathy is achieved by use of combination of ECG and echocardiographic examinations Oyama *et al.,* [1] biomarkers testing have also been reported to clarify the status of dogs with equivocal results when evaluated by other diagnostic modalities [2].

Biochemical marker testing has revolutionized the approach to diagnosis and management of heart failure over the past decade as discovery of new cardiac biomarkers and the increased sensitivity of the assays have extended the boundary of applications [3]. Effective selection of biomarkers requires prospective studies that assess the diagnostic and prognostic value of multiple markers simultaneously. Several studies have demonstrated that multi-marker strategies are worthwhile for providing information of greater value than any single marker alone [4].

2. MATERIALS AND METHODS

The present study was conducted collaboratively at the Department of Veterinary Clinical Medicine, College of Veterinary and Animal Science, RAJUVAS, Bikaner, and the Chandrika Chimanlal Doshi Cardiovascular Unit for Animals, Department of Veterinary Clinical Medicine, Ethics, and Jurisprudence, Bombay Veterinary College, Parel, Mumbai-12. The period of study was from May-2014 to Nov-2014. A comprehensive investigation involving 2497 canines of diverse breeds, ages, and genders was conducted to ascertain occurrences of Dilated Cardiomyopathy (DCM). Through meticulous examination, which encompassed historical analysis, clinical evaluation, electrocardiography, and radiography, researchers definitively diagnosed 29 cases of DCM utilizing echocardiography and cardiac biomarkers. In present study Microelisa Stripplate reader of Erba Lifespan II (Erba Mannheim) and automatic washer (Erba Lisawash, Erba Mannheim) were used. The estimation of biomarkers was performed by the commercially available Quantitative Sandwich ELISA kit. All of which are intended to be determinate concentrations in Canine serum, plasma and other body fluids. Whole procedure was followed as per manufacturer recommendation.

3. RESULTS AND DISCUSSION

Statistical analysis and interpretation of five major canine specific cardiac biomarkers i.e. cardiac Troponin I, B-type natriuretic peptide (BNP), N-Terminal-pro B-type natriuretic peptide (NT- pro-BNP), Atrial natriuretic peptide (ANP) and Endothelin-1 are presented in Table 1.

3.1 Cardiac Troponin I

The present study measured canine-specific cardiac Troponin I (cTnI) levels in healthy dogs and those with DCM. Results showed significant differences between healthy and DCM-affected dogs. Among DCM cases, bilaterally affected dogs had the highest cTnI levels, followed by left DCM, and then right DCM, with undetectable cTnI levels in 5% of cases (Table 1, Fig. 1). Results of cardiac troponin I in healthy dogs were parallel with the findings of Fabio *et al*. [5] who reported 0.20 ng/mL (range <0.20-0.43 ng/mL) concentration in healthy dogs. Melter *et al*. [6] who established reference range of less than 0.3 ng / mL. Range of troponin I obtained in our investigation was much different and lower from Schultze *et al*. [7] who reported less than 2.00 ng/mL as reference value for cTnI in healthy animals with range of 1 to 9 ng/mL In our study 5% healthy dogs (2 out of 40) were reported with undetectable cardiac troponin I concentration, which was lower than Ljungvall *et al*. [8] who reported undetectable concentrations of cTnI in 37 % of the healthy dogs.

Within group analysis for cTnI in present study showed non-significant higher levels of cTnI in bilateral followed by left and right DCM. Possible explanation is that the left ventricle, being part of high pressure system is thicker and work harder to pump blood to supply blood in contrast to right side which is low-pressure system, has a large capacity and high compliance and is often referred to as a blood reservoir. Therefore, pathological changes in myocardium could be more marked in left side dysfunction. Results of previous studies showed wide variations in level of cTnI in both healthy and DCM affected dogs.

Further data mining at three cut off point of c-TnI i.e. 0.3, 0.4 and 0.5ng/dl, showed 96.55 %, 86.20 % and 75.90 % sensitivity and 35 %, 62% and 80 % specificity at these levels, respectively. False negative rates were 30, 14 and 24 % and fall-out rate observed to decrease (65, 38 and 20 %) with increasing cut off levels of cTnI. Positive and negative likelihood ratios were 1.49, 0.10 and 2.29, 0.22 and 3.79, 0.30 at these three cut off levels of cTnI. Diagnostic odds ratios of 15.08, 10.42 and 12.57, precision of 52, 63 and 73 %, negative predictive value (NPV) of 93, 86 and 82 %, false omission rate (FOR) of 7, 14 and 18, false discovery rate (FDR) of 48, 38 and 27 %, accuracy of 28.01, 25.01 and 22.01 and area under curve (AUC) of 0.66, 0.74 and 0.78 was observed for c-TnI at three cut off points. Results of Cohen's Kappa value calculation for agreement of c-TnI with gold standard test showed moderate agreement at 0.4 and 0.5 ng/dl concentrations (Kappa value of 0.463 and 0.556) and poor agreement at 0.3 ng/dl concentration (Kappa value of 0.282).

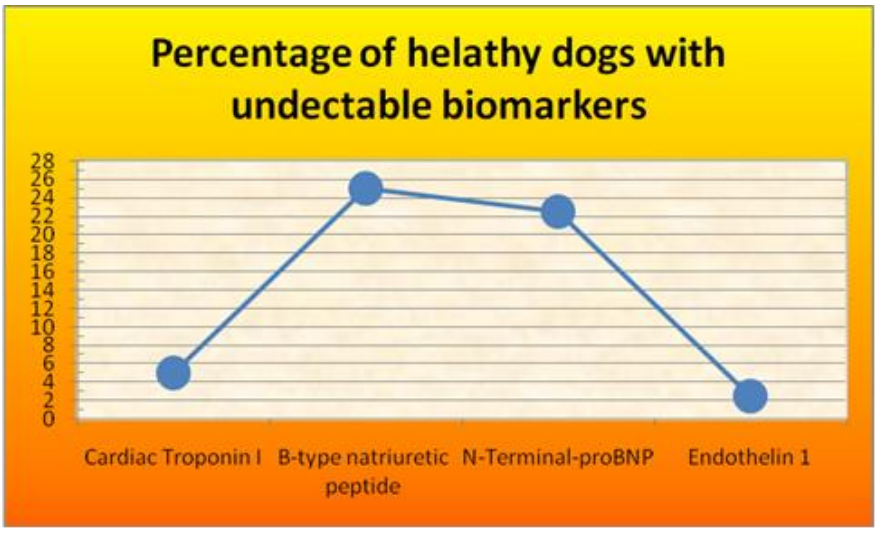

Fig. 1. Percentage of healthy dogs with undetectable biomarkers

Table 1. Test of significance analysis of cardiac biomarkers in healthy and DCM affected dogs

Sl. No.	Biomarker	Healthy	DCM	Left DCM	Right DCM	Bilateral DCM
1.	Cardiac Troponin I (ng/dl)	0.38 ± 0.04 [b] Range = 0.036-0.913 Undetected in 5 % (2 out of 40) dogs	0.72 ± 0.06 [a] Range = 0.136-1.49	0.676 ± 0.057 [a] Range = 0.297-1.027	0.563 ± 0.091 [ab] Range = 0.136-0.867	0.930 ± 0.144 [a] Range = 0.369-1.484
2.	B-type natriuretic peptide (ng/dl)	115.34 ± 16.34 [c] Range = 6.62- 387.39 Undetected in 25 % (10 out of 40) dogs	339.10 ± 45.93 [ab] Range = 27.77-1128.90	340.57 ± 54.38 [bc] Range = 27.77- 846.94	237.02 ± 31.62 [bc] Range = 82.17- 371.55	425.86 ± 125.96 [a] Range = 27.70-1128.90
3.	N-Terminal-proBNP (pmol/l)	109.81 ± 15.86 [b] Range = 11.45- 278.38 Undetected in 22.5 % (9 out of 40) dogs	394.19 ± 77.44 [a] Range = 40.75-1359.98 Undetected in 10.34 % (3 out of 29) dogs)	411.56 ± 127.12 [a] Range = 40.75- 1359.98 Undetected in 7.14 % (1 out of 14) dogs	465.88 ± 92.34 [a] Range = 240.68-896.86 Undetected in 28.57 % (2 out of 7) dogs	321.16 ± 116.54 [ab] Range = 40.74- 980.88
4.	Atrial Natriuretic Peptide (ng/dl)	103.18 ± 8.85 [NS] Range = 15.449- 217.759	336.02 ± 109.09 [NS] Range = 30.815 - 2791.380	380.10 ± 214.23 [NS] Range = 30.815- 2791.380	291.17 ± 122.01 [NS] Range = 54.112-968.596	298.15 ± 113.07 [NS] Range = 63.034-906.127
5.	Endothelin 1 (ng/dl)	[183.33] 15.26 [b] Range = 48.0- 534.34 Undetected in 2.5 % (1 out of 40) dogs	243.82 ± 18.89 [ab] Range = 4.23- 487.49	196.97 ± 6.17 [b] Range = 171.31 -245.56	270.10 ± 39.36 [ab] Range = 189.80- 428.17	302.81 ±52.50 [a] Range = 4.23- 487.49

Table 2- Test performance of Troponin I in healthy and DCM affected dogs

Sl. No.	Parameters	Cut off point of 0.3 ng/dl	Cut off point of 0.4 ng/dl	Cut off point of 0.5 ng/dl
1.	Sensitivity (true positive rate)	96.55	86.20	75.90
2.	Specificity (true negative rate)	35.00	62.50	80
3.	False negative rate	30	14	24
4.	False positive rate (Fall-out)	65	38	20
5.	Positive likelihood ratio	1.49	2.29	3.79
6.	Negative likelihood ratio	0.10	0.22	0.30
7.	Diagnostic odds ratio	15.08	10.42	12.57
8.	Positive predictive value (Precision) (without considering prevalence)	52	63	73
9.	Negative predictive value (without considering prevalence)	93	86	82
10.	False omission rate	7	14	18
11.	False discovery rate	48	38	27
12.	Diagnostic effectiveness (Accuracy)	28.01	25.01	22.01
13.	Area under curve (AUC)	0.66 (sufficient)	0.74 (good)	0.78 (good)
14.	Cohen's Kappa value	0.282 (fair)	0.463 (moderate)	0.556 (moderate)

3.2 B-Type Natriuretic Peptide (BNP)

Results of canine specific B-type natriuretic peptide (BNP) analysis (Table .3) of present investigation revealed significant differences in canine-specific B-type natriuretic peptide (BNP) levels between healthy dogs and those with DCM. Within group analysis revealed non-significant difference between left and right DCM, but dogs with bilateral DCM showed significantly higher level of biomarkers in comparison to left and right DCM. Further data mining at three cut off point of B type natriuretic peptide i.e. 105, 115 and 125 ng/dl, showed 86 %, 83 % and 83 % sensitivity and 65 %, 65% and 78 % specificity at these levels, respectively. False negative rates were found to increase (14, 17 and 17 %) and fall-out rate observed to decrease (35, 35 and 23 %) with increasing cut off levels of BNP. Positive likelihood ratio and negative likelihood ratio were 2.46, 0.21 and 2.36, 0.27 and 3.68, 0.22 at these three cut off levels of BNP. Diagnostic odds ratios of 11.61, 8.91 and 16.53, precision of 64, 63 and 73 %, NPV of 87, 84 and 86 %, FOR of 13, 16 and 14, FDR of 36, 37 and 27 %, accuracy of 25.01, 24.01 and 24.01 and 0.76 , 0.80 and 0.80 area under curve were observed at three cut off points.

Wide variations for BNP level of normal and DCM affected dogs were reported in previous studies. Study of Oyama *et al.* [1] reported 6.51 ± 0.66 and 14.35 ± 1.60 pg/mL BNP concentrations in healthy and DCM affected dogs, respectively.

High BNP in DCM dogs of present study was likely due to increased ventricular synthesis [9]. It has been reported that during early ventricular dysfunction, plasma BNP was increased, and atrial tissue contents of BNP and BNP messenger RNA (mRNA) were also increased.

Present study recorded highest sensitivity of BNP (85.87 %) at 105 and moderate (82.5 %) at 115 and 125 ng/dl concentration. On the other hand, specificity was higher (77.75 %) on 125 ng/ml and moderate (65.45 %) at 115 ng/dl and 110 ng/dl. Although sensitivity and specificity depend on the reference range used, best possible combination of these two seen at 115 ng/dl. Combined accuracy, AUC and Cohen's Kappa value (24.01, 0. 80 and 0.458) were also found comparatively better at 115 ng/dl concentration cutoff point (Table 3 and Fig. 2).

3.3 N-Terminal-Pro-BNP

Results of N-Terminal-pro-BNP analysis revealed (Table.1, Fig. 1) significant difference between healthy and DCM affected dogs with respect to N-Terminal-proBNP. Within group analysis revealed non-significant difference between subgroups of DCM. Further analysis was

performed at three cut off point of NT- pro- BNP i.e. 90, 100 and 110 ng/dl, which showed 79, 79 and 79 % sensitivity and 53, 60 and 63 % specificity at these levels, respectively. False negative rates were constant (21% at all levels) and fall-out rate showed decreasing pattern (48, 40 and 38 %) with increasing cut off levels of NT-pro- BNP. Positive likelihood ratio and negative likelihood ratio were 1.67, 0.39 and 1.98, 0.34 and 2.11, 0.33 at these three cut off levels of BNP. Diagnostic odds ratios of 4.24, 5.75 and 6.39, precision of 55, 59 and 61 %, NPV of 78, 80 and 81 %, FOR of 22, 20 and 19, FDR of 45, 41 and 39 %, accuracy of 23.01 at all levels and 0.66, 0.70 and 0.70 area under curve were observed at three cut off points. Cohen's Kappa values were 0.30, 0.375 and 0.401 at 90, 100 and 110 ng/dl concentrations of this biomarker (Table 4).

Observation made for mean ± SE of healthy dogs in present investigation were in accordance with Noszczyk [10] who reported significant increase NT-pro-BNP (136 Vs 2180 pmol/l) in DCM affected dogs in comparison to healthy dogs.

Compared with the conventional markers (e.g. troponin I), the NT-proBNP has been reported to rise much higher in an earlier phase, making it more suitable for early diagnosis [11] and detection in dogs with various cardiac disorders, such as aortic stenosis, mitral valve disease, and congestive heart failure (CHF) [12].

3.4 Atrial Natriuretic Peptide (ANP)

Results of atrial natriuretic peptide (ANP) analysis revealed (Table.1) non-significant difference between healthy and DCM affected dogs with respect to ANP, within group analysis also revealed non-significant difference in subgroups of DCM. But like troponin I, right DCM group was found to show low level of ANP than left and bilateral DCM. Further analysis was performed at three cut off point of atrial natriuretic peptide i.e. 75, 100 and 125 ng/dl, which showed 72%, 62% and 45 % sensitivity and 27.5%, 55% and 72.5 % specificity at these levels, respectively. False negative rates were found to increase (27.6, 38 and 55.20 %) and fall-out rate observed to decrease (72.5, 45 and 27.5 %) with increasing cut off levels of cTnI. Positive likelihood ratio and negative likelihood ratio were 0.99, 1.003 and 1.379, 0.69 and 1.63, 0.76 at these three cut off levels of cTnI. Diagnostic odds ratios of 1.096, 2.00 and 2.142, precision of 42.0, 50 and 74.4 %, NPV of 57, 66 and 54 %, FOR of 42, 33 and 35, FDR of 58, 50

Table 3. Test performance of BNP in healthy and DCM affected dogs

Sl. No.	Parameters	Cut off point of 105 ng/dl	Cut off point of 115 ng/dl	Cut off point of 125 ng/dl
1.	Sensitivity (true positive rate)	85.87	82.5	82.5
2.	Specificity (true negative rate)	65.45	65.45	77.75
3.	False negative rate	14	17	17
4.	False positive rate (Fall-out)	35	35	23
5.	Positive likelihood ratio	2.46	2.36	3.68
6.	Negative likelihood ratio	0.21	0.27	0.22
7.	Diagnostic odds ratio	11.61	8.91	16.53
8.	Positive predictive value (Precision) (without considering prevalence)	64	63	73
9.	Negative predictive value (without considering prevalence)	87	84	86
10.	False omission rate	13	16	14
11.	False discovery rate	36	37	27
12.	Accuracy	25.01	24.01	24.01
13.	AUC	0.76 (good)	0.80 (good)	0.80 (good)
14.	Cohen's Kappa value	0.489 (moderate)	0.458 (moderate)	0.591 (moderate)

Table 4. Test performance of NT- pro- BNP in healthy and DCM affected dogs

Sl. No.	Parameters	Cut off point of 90 pmol/L	Cut off point of 100 pmol/L	Cut off point of 110 pmol/L
1.	Sensitivity (true positive rate)	79	79	79
2.	Specificity (true negative rate)	53	60	63
3.	False negative rate	21	21	21
4.	False positive rate (Fall-out)	48	40	38
5.	Positive likelihood ratio	1.67	1.98	2.11
6.	Negative likelihood ratio	0.39	0.34	0.33
7.	Diagnostic odds ratio	4.24	5.75	6.39
8.	Positive predictive value (Precision) (without considering prevalence)	55	59	61
9.	Negative predictive value (without considering prevalence)	78	80	81
10.	False omission rate	22	20	19
11.	False discovery rate	45	41	39
12.	Accuracy	23.01	23.01	23.01
13.	AUC	0.66 (sufficient)	0.70 (sufficient)	0.70 (sufficient)
14.	Cohen's Kappa value	0.300 (fair)	0.375 (fair)	0.401 (moderate)

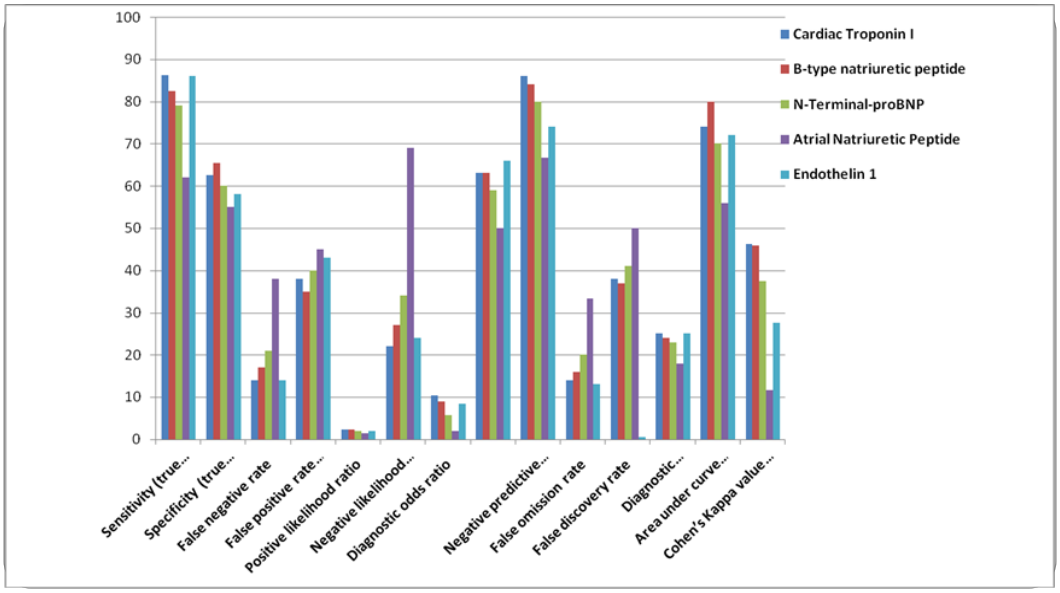

Fig. 2. Compartive analysis of cardiac biomarkers in DCM

and 45.8 %, accuracy of 21.00, 18.00 and 13.01 and 0.50, 1.38 and 0.59 area under curve were observed for atrial natriuretic peptide at three cut off points (Table.5 and Fig. 2).

The results of atrial natriuretic peptide (ANP) in our investigation align with those reported by Asano *et al*. [13] who observed ANP levels of 244.5 and 208.0 pg/ml in two dogs with DCM.

The non-significant findings in DCM affected dogs may be attributed to two factors. Firstly, cardiac production and release of ANP occur relatively early in the course of myocardial disease in dogs, as noted by Oyama et al. [1]. Secondly, ANP is stored as pro-hormones (proANP1-126) in numerous granules within atrial cardiomyocytes, as highlighted by Widmaier *et al*. [14].

Table 5. Test performance of ANP in healthy and DCM affected dogs

Sl. No.	Parameters	Cut off point of 75 ng/dl	Cut off point of 100 ng/dl)	Cut off point of 125 ng/dl
1.	Sensitivity (true positive rate)	72	62	45
2.	Specificity (true negative rate)	27.5	55	72.5
3.	False negative rate	27.6	38	55.2
4.	False positive rate (Fall-out)	72.5	45	27.5
5.	Positive likelihood ratio	0.999	1.379	1.630
6.	Negative likelihood ratio	1.003	0.69	0.76
7.	Diagnostic odds ratio	1.096	2.00	2.142
8.	Positive predictive value (Precision) (without considering prevalence)	42.0	50	64.4
9.	Negative predictive value (without considering prevalence)	57.9	66.7	54.2
10.	False omission rate	42.1	33.3	35.6
11.	False discovery rate	58	50	45.8
12.	Accuracy	21.004	18.009	13.012
13.	AUC	0.50 (fail)	0.56 (fail)	0.59 (fail)
14.	Cohen's Kappa value	-0.001 (worse)	0.116 (poor)	0.177 (poor)

Table 6. Test performance of Endothelin 1 in healthy and DCM affected dogs

Sl. No.	Parameters	Cut off point of 170 ng/dl	Cut off point of 180 ng/dl	Cut off point of 190 ng/dl)
1.	Sensitivity (true positive rate)	93	86	72
2.	Specificity (true negative rate)	45	58	63
3.	False negative rate	7	14	28
4.	False positive rate (Fall-out)	55	43	38
5.	Positive likelihood ratio	1.69	2.03	1.93
6.	Negative likelihood ratio	0.15	0.24	0.44
7.	Diagnostic odds ratio	11.05	8.46	4.38
8.	Positive predictive value (Precision) (without considering prevalence)	55	66	58
9.	Negative predictive value (without considering prevalence)	90	74	76
10.	False omission rate	10	13	24
11.	False discovery rate	45	0.45	42
12.	Accuracy (without considering prevalence)	27.01	25.01	21.01
13.	AUC	0.77 (fair)	0.72 (fair)	0.67 (poor)
14.	Cohen's Kappa value	0.348 (fair)	0.276 (fair)	0.388 (fair)

Although poor, best sensitivity among different cutoff level was seen at 75 ng/dl followed by 100 and 125 ng/dl concentrations. On the other hand best specificity was recorded at cut off level of 125 ng/dl. Previous report of Oyama *et al*. [1] was entirely different from present investigation with respect to both healthy and DCM affected values of ANP, showed 0.658 AUC, 85.7 % sensitivity, 47.4% specificity, PPV of 93.9 and VPV of 26.1 at 7.03 ng/dl cutoff level.

3.5 Endothelin 1

The results of Endothelin 1 analysis (Table 1) revealed non-significant difference between healthy and DCM-affected dogs, as well as between DCM subgroups except for significantly higher levels observed in bilateral DCM to left DCM. Further analysis at three cut-off points of 170, 180, and 190 ng/dl demonstrated sensitivities of 93%, 86%, and 72%, and specificities of 45%, 58%, and 63%, respectively. False negative rates exponentially increased (7%, 14%, and 28%), while the fall-out rate showed a decreasing trend (55%, 43%, and 38%) with increasing Endothelin cut-off levels. Diagnostic odds ratios ranged from 11.05 to 4.38, with corresponding precisions of 55%, 66%, and 58%. Additionally, the area under the curve ranged from 0.77 to 0.72, indicating fair agreement as shown by Cohen's Kappa values of 0.348, 0.276, and 0.388 at these three cut-off points.

Observed level of endothelin 1 in healthy dogs of present study was much higher than previously reported by Tessier-Vetzel *et al*. [15] who reported level of 1·9 ± 0·1 pg/ml.

4. CONCLUSION

In nutshell we can say that the utilization of cardiac biomarkers in diagnosing Dilated Cardiomyopathy (DCM) in dogs presents a promising avenue for early detection, prognosis assessment, and treatment monitoring. This collaborative study underscores the significance of biomarker analysis in veterinary cardiology, demonstrating its potential to augment traditional diagnostic approaches.

The findings highlight significant differences in biomarker levels between healthy dogs and those affected by DCM, particularly in bilaterally affected cases. However, certain biomarkers, such as atrial natriuretic peptide (ANP), showed no substantial variations, emphasizing the need for comprehensive evaluation and the consideration of multiple biomarkers.

Further research involving larger dog populations is warranted to validate and refine these findings, ensuring the broader adoption of biomarker testing in veterinary practice.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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