

Evaluation of plasmatic levels of CK-MB and C-Reactive Protein in brachicephalic dogs who have narin stenosis before and after diode laser rhinoplasty

Avaliação dos níveis plasmáticos de CK-MB e Proteína C-Reativa em cães braquicefálicos portadores de estenose de narinas antes e após rinoplastia com laser de diodo

Evaluación de niveles plasmáticos de CK-MB y proteína C-Reactiva en perros braquicefálicos con estenosis de las fosas nasales antes y después de la rinoplastia con láser de diodo

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Abstract

To evaluate the protective effect of rhinoplasty with diode laser on the cardiovascular system and in the reduction of the inflammatory state, CK-MB and PCR measurements were performed before and 30 days after the surgical procedure. Rhinoplasty using the diode laser promoted a significant reduction in plasma CK-MB and PCR levels, 30 days after its completion. No association was found between the pre-surgical plasma concentration of CK-MB and CRP and the degree of stenosis of the nostrils, race, age, sex and weight of the animals. A significant reduction in CK-MB was observed in dogs with severe stenosis, French Bulldog, one year old and over four years old, male and between 10 and 20 kg. Animals with severe stenosis, of the Pug breed, weighing less than 10 kg and three years of age exhibited a significant decrease in serum CRP values after surgery. As a result, the degree of stenosis of the nostrils, the breed, age, sex and weight of the animals did not influence the plasma concentration of NTpro-BNP, CK-MB and PCR in brachycephalic dogs. Rhinoplasty with diode laser has been shown to be effective in reducing the plasma concentration of the natriuretic peptide CK-MB and CRP in dogs with brachycephalic syndrome, showing that the

procedure has a protective effect on the cardiovascular function of these animals and is also effective in reducing inflammatory biomarkers.

Keywords: Biomarkers; Inflammation; Obstructive syndrome.

Resumo

Com o objetivo de avaliar o efeito protetivo da rinoplastia com laser de diodo no sistema cardiovascular e na diminuição do estado inflamatório, foi realizada dosagem de CK-MB e PCR antes e 30 dias depois do procedimento cirúrgico. A rinoplastia com o uso do laser de diodo promoveu redução significativa nos níveis plasmáticos CK-MB e PCR. Não foi constatada associação entre a concentração plasmática pré-cirúrgica de CK-MB e PCR e o grau de estenose de narinas, raça, idade, sexo e peso dos animais. Redução significativa de CK-MB foi observada em cães com estenose de narinas grave, da raça Buldogue francês, de um ano e acima de quatro anos de idade, machos e entre 10 e 20kg. Animais com estenose grave, da raça Pug, com menos de 10kg e três anos de idade exibiram decréscimo significativo nos valores séricos de PCR pós cirurgia. A rinoplastia com laser de diodo mostrou-se efetiva na redução da concentração plasmática do peptídeo natriurético CK-MB e PCR em cães portadores da síndrome braquicefálica, mostrando que o procedimento exerce efeito protetivo na função cardiovascular desses animais sendo eficaz ainda na diminuição dos biomarcadores inflamatórios.

Palavras-chave: Biomarcadores; Inflamação; Síndrome obstrutiva.

Resumen

Con el fin de evaluar el efecto protector de la rinoplastia con láser de diodo sobre el sistema cardiovascular y en la reducción del estado inflamatorio, se realizaron mediciones de CK-MB y PCR antes y 30 días después del procedimiento quirúrgico. La rinoplastia con el uso de láser de diodo promovió una reducción significativa de los niveles plasmáticos de CK-MB y PCR. No se encontró asociación entre la concentración plasmática prequirúrgica de CK-MB y PCR y el grado de estenosis de las fosas nasales, raza, edad, sexo y peso de los animales. Se observó una reducción significativa de CK-MB en perros con estenosis severa de las fosas nasales, de la raza Bulldog Francés, de un año y más de cuatro años, machos y entre 10 y 20 kg. Los animales con estenosis severa, de la raza Pug, con un peso inferior a 10 kg y tres años, mostraron una disminución significativa en los valores de PCR sérica después de la cirugía. La rinoplastia con láser de diodo fue eficaz para reducir la concentración plasmática del péptido natriurético CK-MB y la PCR en perros con síndrome braquicefálico, demostrando que el procedimiento tiene un efecto protector sobre la función cardiovascular de estos animales, siendo eficaz incluso en la reducción de biomarcadores inflamatorios.

Palabras clave: Biomarcadores; Inflamación; Síndrome obstrutiva.

1. Introduction

The brachycephalic syndrome is directly linked to obstructive processes that occur due to important anatomical deformities of the anterior airways (Honey, 2017; Packer, O'Neill, Fletcher, Farnworth, 2019) These changes impact the physiological functions of the respiratory, cardiovascular and digestive systems, significantly compromising the well-being and quality of life of these animals (Fawcett et al., 2019).

It is known that the earlier the resistance to air entry, the greater the inspiratory effort (Koch, Arnold, Hubler, Montavon, 2003; Meola, 2013). Bilateral nostril stenosis, being the most anterior anatomical alteration, is an important point of airway obstruction, strongly increasing inspiratory overload (Koch, Arnold, Hubler & Montavon, 2003)

It is believed that cardiovascular changes may appear in dogs brachycephalic disease secondary to obstructive disease. Thus, clinical signs, such as syncope, arrhythmias, cyanotic mucous membranes, dyspnea and exercise intolerance can be found (Camacho, 2006). Humans diagnosed with obstructive sleep apnea are susceptible to hypertensive episodes, due to greater sympathetic activity, greater endothelial damage, decreased nitric oxide production and increased intrathoracic pressure negative, causing an increase in blood pressure (Gonzaga et al., 2015). It is considered that, due to the respiratory syndrome, these episodes may occur in the brachycephalics (Hoffman, 2007).

In accordance with the obstructive pathophysiological process and the progressive and chronic character of the disease, brachycephalic dogs are believed to be highly susceptible to the occurrence of pulmonary hypertension and, therefore,

to the development of Cor Pulmonale (Hoareau & Mellena, 2012), due to chronic alveolar hypoxia and the consequent pre-capillary arterial vasoconstriction, which leads to increased pulmonary artery pressure and possible right ventricular hypertrophy (Allen & Mackin, 2001, Fasanella, Shivley, Wardlaw & Givaruangsawat, 2010). Of extreme relevance among cardiovascular diseases to which brachycephalics are predisposed, pulmonary hypertension is highlighted by the reserved prognosis, and early diagnosis is important, with the aim of improving the quality of life and increasing the survival of these animals (Hoareau & Mellena, 2012).

The rhinoplasty is the surgical procedure that aims to modify the shape or size of the nose (Lodato & Hedlund, 2012). In brachycephalics with stenosis of the nostrils, this procedure aims at opening the most anterior portion of the nostril, which causes the obstruction. It is a simple and easy to perform technique (Dupré & Heidenrich, 2016), with minimal surgical risk for the animal (Tarricone, Hayes, Singh & Davis, 2019).

The diode laser is a low cost equipment when compared to other laser emitters, such as CO₂. The laser interacts with the target tissue and, in addition to performing the ablation, has a biostimulating action and seals blood, lymph vessels and nerve endings, thereby providing better hemostasis, reducing the need for sutures and shortening the surgical time. In the trans and postoperative period, there is a lower rate of edema, inflammation and pain (Dunié-Mérogot; Bouvy; Poncet, 2010).

Due to the predisposition to the development of changes cardiovascular diseases secondary to anterior airway obstruction, periodic cardiovascular evaluation is recommended in these animals, in addition to gold standard exams, such as echocardiogram and electrocardiogram. The CK-MB biomarkers, in addition to the biomarker of inflammatory changes in C-reactive protein (CRP), have an important predictive value in the detection of lesions in this organ (Polizopoulou et al., 2015; Sadanaga, 2018).

In this sense, this work proposes to evaluate, through the dosage of cardiac and inflammatory biomarkers before and after laser rhinoplasty, the protective effect of this procedure on the cardiovascular function of brachycephalic dogs.

2. Material and Methods

This project was approved by the Ethics Committee on the Use of Animals at The Federal Fluminense University, under protocol number 960/2018.

The present quantitative experimental study (Pereira et al. 2018), was carried out at the Veterinary Faculty of the Universidade Federal Fluminense (UFF), Niterói, Rio de Janeiro, Brazil. Clinical evaluations and rhinoplasty procedures, performed at the University Hospital of Veterinary Medicine Professor Firmino Mársico Filho (HUVET-UFF). The blood samples collected were stored at the Poultry Health Laboratory (MSV-UFF).

Measurements of C-reactive protein (PCR) and creatinophosphokinase - MB (CK-MB) were performed at the Research Laboratory in Veterinary Clinical Pathology and Molecular Biology Marsílio Dias do Nascimento (LAMADIN - UFF).

Were included to the study, brachycephalic dogs, male or female, aged one year or more, with bilateral stenosis of the nostrils, regardless of the other obstructive anatomical changes common to the brachycephalic syndrome. All presented clinical and complementary exams exempt from systemic changes not directly related to brachycephalics.

Were excluded Animals under one year of age or who had respiratory disorders diagnosed on radiographic examination, primary cardiac disorders diagnosed on echocardiographic and electrocardiographic examinations were excluded from the study, except Cor Pulmonale; in addition to animals infected with *Dirofilaria immitis*, or that had hematological and / or biochemical changes in serum, liver and kidneys that made it impossible to subject the animal to surgical or anesthetic procedures.

The biomarkers CK-MB e PCR, were measured in the preoperative phases (on the day of surgery) and 30 days after the rhinoplasty.

For each evaluation, a sample of approximately 10mL of venous blood was obtained by venipuncture of the jugular vein. For this procedure, after trichotomy and asepsis of the region, 10mL hypodermic syringes and 25 X 7mm needles were used.

The samples obtained at different times were equally divided into a 5mL aliquot of blood, packed in a tube without anticoagulant, for the removal of blood serum and subsequent measurement of cTnI, PCR and CK-MB.

The volume of blood collected was immediately separated into serum and blood plasma in a standard centrifuge, with a rotation of 1500G for 10 minutes, and transferred with the aid of a pipette to 2mL plastic conical tubes. The tubes were accommodated in an identified tray and stored in a freezer with a controlled temperature of -70°C in the Laboratory of Poultry Health (MSV-UFF), for later dosage of biomarkers. The samples were always obtained in duplicate.

Plasma measurement of the MB fraction of Creatinophosphokinase was performed using the kinetic method in blood serum, using the CK-MB Liquiform® kit (Labtest), equal to or less than 200 U/L.

Blood serum was also used to quantify C-reactive protein by immunoturbidimetry with the Turbiquest Plus® PCR kit (Labtest). The samples were dosed using the automatic analyzer LabMax® 240 Premium® (Labtest) at the LAMADIN-UFF Laboratory, Niterói, RJ. The quantitative reference values adopted for cardiac risk were: less than 1mg / L, low risk; from 1 to 3mg / L, moderate risk; higher than 3mg / L high risk.

As pre-anesthetic medication, nalbuphine hydrochloride (0.5mg / kg) was used, intramuscularly, and induction, propofol (3mg / kg) associated with midazolam hydrochloride (0.3mg / kg), intravenously. Maintenance was performed using the inhalation technique with 1.5% isoflurane. During the trans-surgical period, for analgesia, sodium dipyrone (25mg / kg) was administered and, as an anti-inflammatory, dexamethasone disodium phosphate (0.5mg / kg), both intravenously.

After performing asepsis in the surgical region, the patient was placed in prone position with the head slightly raised. The facial area was covered with a field cloth, showing only the nostril region. The surgical procedure was always performed by the same surgeon, using a DMC Thera lase Surgery® device (DMC Import and Export Equipment) with diode laser and infrared wavelength $808\text{nm} \pm 10\text{nm}$ and useful power of $9\text{W} \pm 20\%$, with fiber diameter $400\mu\text{m}$.

In the rhinoplasty, the technique of alavestibuloplasty was performed, the laser was used in the continuous mode at the power of 2.8W, proceeding to the internal and external marking of the wings of the nostrils to be removed and, then, the photoablation was performed through the medial portion until the caudal portion of the hypertrophied wing, thus releasing the opening of the nasal cavity (Figure 1E, F).

After the end of the procedure, the supply of Isoflurane was interrupted and the animal was awakened and then released, after full anesthetic recovery, with the recommendation to use the cervical collar and schedule the return in 15 and 30 days, for evaluation of the surgical wound.

The collected data were tabulated in Microsoft Excel 2010® and all statistical analyzes were performed using the BioEstat 5.3 program (Ayres et al., 2007).

The results of the biomarkers were submitted to the normality test (Shapiro-Wilk) and compared between the evaluation moments 0 and 30 days using the Wilcoxon Test. The relationship between variables was analyzed using Pearson's correlation test. In categorical comparisons between groups, the Mann Whitney and Kruskal Wallis tests were used, when necessary.

All analyzes were performed using the BioEstat 5.3 program (Ayres et al., 2007).

3. Results

Thirty brachycephalic dogs with stenosis of the nostrils were evaluated, 16 (53.3%) males and 14 (46.7%) females, 81.2% of males and 50% of females were not neutered. The average age of the animals was 2.9 years (ranging from 1 to 9 years), with an average weight of 11.3 kg (ranging from 8 to 14 kg). Regarding breeds, 19 (63.3%) dogs were French Bulldog and 11 (36.7%) were Pugs.

Regarding the degree of stenosis of the nostrils, of the 30 animals in the study, six (20%) exhibited stenosis of the nostrils classified as grade 2 (moderate stenosis of the nostrils) and 24 (80%) as grade 3 (severe stenosis of the nostrils).

The mean values of plasma concentrations of CK-MB AND PCR biomarkers before and 30 days after rhinoplasty of the 30 evaluated brachycephalic dogs, are described in Table 1.

Table 1 - Descriptive analysis and comparison of the means by the Wilcoxon test of the values of CK-MB and PCR, of the 30 brachycephalic dogs with stenosis of the nostrils, before and 30 days after rhinoplasty with diode laser.

Variable	Before rhinoplasty					After rhinoplasty				
	n	Mean	SD	Min.	Max.	n	Mean	DP	Min.	Max.
CK-MB (U/L)	30	147.28 ^a	±53.38	72.8	285	30	104.9 ^b	±42.02	7	216.8
PCR (mg/L)	30	0.25 ^a	±0.21	0	0,7	30	0.12 ^b	±0.16	0	0.6

* Letters (a / b) equal within the same variable do not differ statistically (P> 0.05 not significant at 5%). n = number of animals; SD = standard deviation; Min. = Minimum; Max. = Maximum. Source: Authors.

Initially, an average of 147.28 U / L was found and after the surgical procedure, 104.93 U / L, a decrease of 42.35 U / L (p <0.0001), with a significant reduction in the plasma concentrations of the 30 brachycephalic dogs being evidenced 30 days after the rhinoplasty.

Animals with a moderate degree of stenosis had a higher mean plasma CK-MB concentration (182.97 U / L) than those with severe stenosis (138.35 U / L), but without statistical significance. The results of the analysis of the association of the different variables with the means of the cardiac biomarker CK-MB before the animals were submitted to rhinoplasty with diode laser are shown in Table 2.

Table 2 - Descriptive analysis and association of CK-MB means by the Wilcoxon test of the different variables of the 30 brachycephalic dogs with stenosis of the nostrils, before undergoing rhinoplasty with diode laser.

Variable		n	CK-MB (U/L) Before rhinoplasty	P value
Degree of stenosis	Moderate	6/30	182.97±51.77 ^a	0.0519
	Severe	24/30	138.35± 50.93 ^a	
Breed	French bulldog	19/30	137.08±42.85 ^a	0.3893
	Pug	11/30	164.88±66.47 ^a	
Age	1 year	8/30	158.09±29.03 ^a	0.3363
	2 years	7/30	162.26±66.00 ^a	
	3 years	6/30	138.18±77.64 ^a	
	> 4 years	9/30	132.08±43.84 ^a	
Sex	Whole male	13/30	147.97±61.22 ^a	0.9572
	Neutered male	3/30	148.20±69.39 ^a	
	Whole female	7/30	157.86±63.23 ^a	
	Neutered female	7/30	135.01±19.08 ^a	
Weight	Up to 10kg	10/30	133.17±33.88 ^a	0.4283
	10-20kg	20/30	153.32±59.53 ^a	

n = number of animals. * Letters (a / b) equal within the same variable do not differ statistically (P> 0.05 not significant at 5%). Source: Authors.

Significant reduction was observed in animals with a greater degree of nasal stenosis. These after rhinoplasty showed an average CK-MB of 91.00 U / L, a reduction of 47.35 U / L (p = 0.0001), while those with moderate stenosis reduced 22.33 U / L (p = 0, 3454).

The results of the analysis of the association of the different variables with the averages of the cardiac biomarker CK-MB, before and after the animals were submitted to rhinoplasty with diode laser are shown in Table 3.

Table 3 - Descriptive analysis and association of CK-MB means by the Wilcoxon test with the different variables of brachycephalic dogs with stenosis of the nostrils, before and after rhinoplasty with diode laser.

Variable	n	CK-MB (U/L)			P value	
		Before rhinoplasty	After rhinoplasty	Reduction 30 days after rhinoplasty		
Degree of stenosis	Moderate	6/30	182.97±51.77 ^a	160.63±42.13 ^a	22.33±45,82	0.3454
	Severe	24/30	138.35± 50.93 ^a	91.00±28.78 ^b	47.35±52,04	0.0001
Breed	French bulldog	19/30	137.08±42.85 ^a	100.95±45.60 ^b	36.13±22,56	0.0001
	Pug	11/30	164.88±66.47 ^a	111.79±35.99 ^a	53.09±80,38	0.0754
Age	1 year	8/30	158.09±29.03 ^a	107.03±26.02 ^b	51.06±12,01	0.0117
	2 years	7/30	162.26±66.00 ^a	118.83±77.0 ^a	43.43±67,90	0.0910
	3 years	6/30	138.18±77.64 ^a	89.30±21.74 ^a	48.88±83,11	0.1159
	> 4 years	9/30	132.08±43.84 ^a	102.67±26.51 ^b	29.41±35,69	0.0109
Sex	Male	16/30	148.01±60.33 ^a	91.14±33.62 ^b	56.87±55,08	0.0004
	Female	14/30	146.44±46.41 ^a	120.68±46.18 ^a	25.76±42,10	0.0555
Weight	Up to 10kg	10/30	133.17±33.88 ^a	104.38±29.42 ^a	28.79±39,00	0.0663
	10-20kg	20/30	153.32±59.53 ^a	105.16±47.05 ^b	48.16±55,34	0.0005

n = number of animals. * Letters (a / b) equal within the same variable do not differ statistically (P> 0.05 not significant at 5%). Source: Authors.

The French Bulldog breed initially showed lower levels of CK-MB (137.08 U / L) when compared to Pugs (164.68 UL). After rhinoplasty in bulldogs, a significant decrease in the biomarker 100.95 U / L (p = 0.0001) was verified in this breed.

In relation to the age of the animals, younger dogs expressed higher CK-MB levels, with a significant decrease being observed in animals of one and above four years of age. In males, there was also a statistical difference in the CK-MB concentration before and after rhinoplasty ($p = 0.0004$).

Dogs weighing over 10 kg, had higher serum CK-MB levels (153.32 U / L), when compared to animals with lower body weight (133.17 U / L) ($p = 0.4283$). In the post-rhinoplasty measurement, there was a significant decrease in the concentration of CK-MB in animals between 10 and 20 kg.

Significant decrease in the mean CRP concentration of the 30 dogs brachycephalic after performing rhinoplasty with diode laser was also noticed. Before the procedure, an average of 0.25 mg / L was measured and 30 days after surgery 0.12 mg / L , a decrease of 0.13 mg / mL ($p = 0.0203$).

Animals with a more severe degree of stenosis of the nostrils showed higher averages of CRP (0.27 mg / L), compared to those with moderate stenosis (0.17 mg / L), but no statistical significance was found. The results of the analysis of the association of the different variables with the means of the PCR before the animals were submitted to rhinoplasty with diode laser are shown in Table 4.

Table 4 - Descriptive analysis and association of OCR means by the Wilcoxon test of the different variables of the 30 brachycephalic dogs with stenosis of the nostrils, before undergoing rhinoplasty with diode laser.

Variable	n	PCR (mg/L) Before rhinoplasty	P value
Degree of stenosis	Moderate	$0.17 \pm 0,14^a$	0.3246
	Severe	$0.27 \pm 0,23^a$	
Breed	French bulldog	$0.24 \pm 0,21^a$	0.7604
	Pug	$0.27 \pm 0,23^a$	
Age	1 year	$0.20 \pm 0,24^a$	0.7396
	2 years	$0.21 \pm 0,12$	
	3 years	$0.32 \pm 0,24^a$	
	> 4 years	$0.28 \pm 0,24^a$	
Sex	Whole male	$0.23 \pm 0,20^a$	0.1269
	Neutered male	$0.3 \pm 0,06^b$	
	Whole female	$0.36 \pm 0,17^a$	
	Neutered female	$0.27 \pm 0,26^a$	
Weight	Up to 10kg	$0.37 \pm 0,26^a$	0.0859
	10-20kg	$0.20 \pm 0,18^a$	

n = number of animals. * Letters (a / b) equal within the same variable do not differ statistically ($P > 0.05$ not significant at 5%). Source: Authors.

After rhinoplasty, a statistically significant difference was observed in the post-surgical plasma levels of this marker in animals with severe stenosis, whereas in animals with moderate stenosis, statistical significance was not found after rhinoplasty.

The results of the analysis of the association of the different variables with the averages of the cardiac biomarker PCR, before and after the animals were submitted to rhinoplasty with diode laser are shown in Table 5

Table 5 - Descriptive analysis and association of PCR means by the Wilcoxon test with the different variables of brachycephalic dogs with stenosis of the nostrils, before and after rhinoplasty with diode laser.

Variable	n	PCR (mg/L)			P value	
		Before rhinoplasty	After rhinoplasty	Reduction 30 days after rhinoplasty		
Degree of stenosis	Moderate	6/30	0.17±0.14 ^a	0.23±0.23 ^a	-0.07±0.15	0.2850
	Severe	24/30	0.27±0.23 ^a	0.10±0.12 ^b	0.18±0.25	0.0058
Breed	French bulldog	19/30	0.24±0.21 ^a	0.13±0.17 ^a	0.11±0.27	0.1401
	Pug	11/30	0.27±0.23 ^a	0.11±0.14 ^b	0.16±0.23	0.0499
Age	1 year	8/30	0.20±0.24 ^a	0.14±0.15 ^a	0.06±0.28	0.5896
	2 years	7/30	0.21±0.12 ^a	0.16±0.21 ^a	0.06±0.19	0.4185
	3 years	6/30	0.32±0.24 ^a	0.07±0.12 ^b	0.25±0.21	0.0431
	> 4 years	9/30	0.28±0.24 ^a	0.12±0.15 ^a	0.16±0.30	0.1282
Sex	Male	16/30	0.19±0.20 ^a	0.10±0.13 ^a	0.09±0.23	0.1361
	Female	14/30	0.31±0.22 ^a	0.15±0.18 ^b	0.16±0.28	0.0528
Weight	Up to 10kg	10/30	0.37±0.26 ^a	0.10±0.13 ^b	0.27±0.30	0.0423
	10-20kg	20/30	0.20±0.18 ^a	0.12±0.17 ^a	0.07±0.22	0.1823

n = number of animals. * Letters (a / b) equal within the same variable do not differ statistically (P> 0.05 not significant at 5%). Source: Authors.

Pug dogs initially showed a mean CRP of 0.27 mg / L and a French Bulldog of 0.24 mg/L. After rhinoplasty the averages decreased to 0.13 mg / mL for French Bulldogs and 0.11 mg / L for Pugs. Statistical significance was found in the reduction of post-surgical PCR values in the Pug breed.

With regard to weight and age, rhinoplasty promoted a significant reduction in CRP in animals with less than 10 kg of body weight and in dogs with 3 years of age.

4. Discussion

Despite being less specific for detecting cardiac lesions than other biomarkers, such as troponin, the MB fraction of creatinophosphokinase has a revealing diagnostic value, when associated with other methods (Silva & Moresco, 2011). All 30 animals evaluated showed CK-MB values within the normal range before the rhinoplasty procedure. A significant difference was found in CK-MB concentrations before and after surgery, showing that rhinoplasty with diode laser was effective in decreasing this biomarker in brachycephalic dogs.

Dogs with severe degree of stenosis showed a significant decrease in the mean plasma concentration of CK-MB after surgery, reinforcing the fact that animals with a more severe degree of obstruction are predisposed to the development of changes secondary to the disease, due to the reduction in air space, which makes it difficult for air to reach the lungs (Liu et al, 2016).

French Bulldog dogs showed lower levels of CK-MB, when compared to Pugs, and a significant decrease in biomarker after surgery. Studies show that Pug dogs have a shorter skull, in addition to a greater number of obstructive changes compared to French Bulldogs (Oechtering; et al., 2016). Thus, it is understood - reduction in CKMB after surgical correction of nostril stenosis is greater in animals with a less marked degree of brachycephaly, as seen in the French Bulldogs of this research.

Unexpectedly, although no significant difference was observed, younger dogs exhibited higher levels of CK-MB, which can be justified by the fact that, due to the intense selection, the animals manifest clinical symptoms earlier and earlier (Planellas et al., 2015). However, other studies seem to be necessary, with a larger number of animals in this age group.

Similarly to what was observed with the levels of NT-proBNP, a significant decrease was observed post-rhinoplasty in the plasma concentrations of CK-MB in males and in animals of greater body weight, probably due to the decrease in the obstruction intensified by muscle mass and by fat.

Despite being less specific for the detection of cardiac injuries, the CK-MB showed diagnostic value in the research, which is why its measurement can be useful as an auxiliary method of diagnosis and prognosis of cardiac injury, when associated with routine cardiovascular assessment.

In the current study, all animals evaluated (100%) showed plasma CRP levels below 1mg / L of blood, with an average of 0.25mg / L, values considered within the normal range. The occurrence of an inflammatory state was not explained, despite the suggestion that, as a result of the different obstructions in the anterior airway, brachycephalic dogs, similar to humans affected by obstructive sleep apnea syndrome, develop hypoxia and hypoxemia (Gianella, et al., 2019), increasing fatigue and oxidative stress in the muscles and soft tissues, which would lead to a chronic chronic inflammatory condition (Chandrashekara, 2014).

The results raised in the study now conducted are in agreement with the report by Planellas et al. (2012), in which the authors evaluated the plasma levels of different acute phase proteins in brachycephalic dogs, and concluded that 85% of the animals did not have high concentrations of C-reactive protein. That finding was confirmed in 2015 by Planellas, et al., when comparing the plasma levels of CRP in brachycephalic dogs before and after multiple corrective surgery for obstructive syndrome and found that 91% of the animals had normal CRP values, which shows that dogs brachycephalics do not develop a state of persistent inflammation.

Later, Gianella et al. (2019) reported that 14% of brachycephalic dogs admitted to their study exhibited C-reactive protein concentrations above normal values, however the authors found no statistical significance in the severe clinical signs presented by the animals.

In the present study, there was a significant reduction in the mean CRP concentration of the 30 brachycephalic dogs after performing the rhinoplasty with diode laser. Before the procedure, an average of 0.25mg / L was measured, and 30 days after surgery, 0.12mg / L, a decrease of 0.13mg / mL.

Usually in human beings with obstructive sleep syndrome, there are increased values of C-reactive protein, with a significant reduction in the levels of this biomarker in adult patients after surgical treatment (Kang et al., 2020). Although brachycephalic dogs do not develop an inflammatory condition as expressive chronic as humans with anterior airway obstruction, rhinoplasty with diode laser was able to decrease C-reactive protein levels.

However, in studies carried out on brachycephalic dogs, despite the reduction in plasma levels of CRP, no statistical significance was found, before and after the completion of multiple surgical corrections of brachycephalic syndrome, suggesting, to elucidate these data, a follow-up to long term (Planellas, et al., 2015, Gianella et al., 2019).

Animals with a more severe degree of stenosis of the nostrils showed higher averages of CRP compared to those with moderate stenosis, but there was no statistical significance. The data were similar to those of Planellas et al. (2012), who also found higher levels of CRP in brachycephalic dogs with greater anterior airway obstruction.

After rhinoplasty, a statistical difference in the plasma levels of this marker was observed in animals with severe stenosis, reinforcing that, because it is the most anterior anatomical alteration, stenosis of the nostrils is an important point of

airway obstruction, strongly increasing inspiratory overload and exerting a direct impact on the degree of airway inflammation (Koch, Arnold, Hubler, Montavon, 2003; Liu, Sargan, Adams & Ladlow, 2015).

In contrast to the current study, in which Pug dogs initially showed mean CRP concentrations of 0.27mg / ml and French Bulldogs of 0.24mg / ml, and after rhinoplasty, the means decreased to 0.10 mg / L for Pugs and 0.13 for French Bulldogs. Planellas et al. (2012) evaluated different inflammatory markers in brachycephalic dogs and pointed out that French Bulldog dogs had higher mean levels of CRP than Pugs, however in both studies there was no statistical significance between the breed of animals and the concentration of CRP, which shows that specific characteristics of the different breeds do not directly influence the serum levels of this inflammatory biomarker in brachycephalic dogs.

A significant difference was found in the reduction in the post-surgical values of Pug dogs, which reinforces the suggestion that, due to the intense brachycephaly, these animals may develop more serious secondary disorders arising from the inflammation of the airways by primary changes. Studies show that Pug dogs have a shorter skull than French Bulldogs as well as the smaller pharyngeal space, when compared to other brachycephalic breeds (Caccamo et al., 2014), in addition to other obstructive changes, such as a high incidence of aberrant turbinates and deviated septum (Oechtering et al., 2016). Thus, it is understood that inflammatory lesions in the Pug are generally more accentuated than in the French Bulldog, justifying the significant reduction of this marker, due to the opening of the nostrils and, consequently, reduction of inspiratory overload.

Similarly, the average age of the animals evaluated by Planellas et al. (2012) and Planellas et al. (2015), which was 2.5, and Gianella et al. (2019), three years, the average age group of dogs admitted here was 2.9 years old. Significant post-surgery reduction was observed in three-year-old animals.

It is suggested that in human patients with obstructive sleep syndrome, age is a risk factor for the disease, since it is diagnosed regularly in adult patients (Kang et al., 2020). In brachycephalic dogs, the severity of the condition is directly linked to the obstructive components, and clinical manifestation is also common in very young animals due to the intense selection to which they were submitted. As a result, it is believed that the chronic inflammatory process determined by advanced age is more evident in human patients than in brachycephalic dogs (Planellas et al., 2012).

It is worth noting that several studies corroborate that brachycephalic syndrome is a progressive disease (Dupré & Heidenrich, 2016; Packer et al., 2019), which is why it is possible to directly relate age to the onset of secondary changes that would induce the inflammatory process, causing older animals to manifest more severe clinical signs.

4. Conclusion

The degree of stenosis of the nostrils, the breed, age, sex and weight of the animals, did not influence the plasma concentration of CK-MB and PCR in brachycephalic dogs.

Rhinoplasty with diode laser is effective in reducing the plasma concentration of the CK-MB and PCR in dogs with respiratory obstructive syndrome, showing that the procedure has a protective effect on the cardiovascular function of these animals.

For more information about the influence of the variables evaluated and the concentration of CK-MB and PCR in brachycephalic dogs, studies are suggested evaluating the serum levels of these biomarkers in the long term after performing rhinoplasty with diode laser.

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