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Intestinal Permeability Targeted Rectal Enema Nutraceutical Intervention in Dogs with Cutaneous Adverse Food Reactions: Gut-Brain-Skin Axis Directed Pro-active Treatment

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Abstract

Inflammatory cutaneous disorders have frequently been subjected to treatment trials with very limited success by pharmaceutical intervention, such as corticosteroids and non-steroidal anti-inflammatory drugs. Nevertheless, the latter drugs might be usual suspects for undesirable side effects. In an attempt to overcome those side effects, natural nutraceuticals are being investigated by the present researchers group. In this scenario, 5 different set of nutraceutical interventions [were numbered by their relevant usage order as Nutr-i1 to Nutr-i5] via rectal enema was deemed available at the present study as a novel protocol for pro-active treatment of cutaneous adverse food reactions (caFr) among dogs involved. In a total of 10 dogs with caFr, at the age of 2 to 7 years old, of both sexes, from various breeds diagnosis was based on i) in vitro serum testing for selected/specific IgE ii) intradermal testing different antigens, iii) elimination diet trial and iv) treatment based respond evaluation. Pruritus, as a vast majority presented clinical sign, scoring deemed available visual analog scale (pVaS). Mean ($\bar{X}$ ± Sd) pVaS scores showed significant decreases after treatment in contrast to prior values [1.4 ± 1.27 vs.7.7 ± 1.62, respectively ($p<0.005$)]. As discussed herein in the whole manuscript, all Nutr-i1 to Nutr-i5 protocols showed pro-active treatment efficacy, which could contribute to novel protocol establishment.

Keywords: Inflammatoric cutaneous disorders, nutraceutical, phytotherapy, polyphenol, probiotic.

INTRODUCTION

Cutaneous adverse food reactions (caFr) is one of the foremost diagnoses frequently detected in dogs/cats with allergic diseases (Olivry and Mueller, 2017). The latter caFr might mimic either noncutaneous (Mueller and Olivry, 2017) or cutaneous clinical signs. Given itching is generally regarded as the vast majority of clinical finding affecting pets with a caFr (Shimakura and Kawano, 2021), consensus/accepted guidelines on caFr in dogs and cats are lacking.

Pathogenesis of caFr is generally based on hypersensitivity and food antigen that stimulate to the immune system considering related studies (Picco et al., 2008; Nemser et al., 2014; Mueller and Unterer, 2018). Unlike humans, there is an indistinguishable anamnesis and clinical background between caFr and atopic dermatitis that usually confusing (Hillier and Griffin, 2001; Olivry et al., 2007). Some studies showed that the pathophysiology of caFr and atopic dermatitis were shown similarities due to increased food-specific immunoglobulin in dogs with atopic dermatitis and gastrointestinal disease (Foster et al., 2003; Pucheu-Haston et al., 2020).

Increased mucosal permeability in dogs with gastrointestinal tract disease is caused by increased antigenic stimulation. Indeed, it is known that food-specific IgG levels are increased in those dogs (Foster et al., 2020). Most of the studies demonstrate a close relation of gut–skin axis in dogs (Craig, 2016; De Pessemier et al., 2021; Rostaher et al., 2022; Ural, 2022). The gut microbiome as breeding of the immune system has the ability to tolerate and avoiding from allergies (Salzman, 2014; Aitoro et al., 2017; Wang et al., 2021; Augustine et al., 2022). Hence the probiotic could be capable of regulating the immune system [via changing gut microbiota and leading to the mucosal/systemic immune defense by modification of cytokine releasing and intestinal IgA responses]. They concurrently modulate to Th1/Th2 balance by suppressing the Th2 response and stimulating the Th1 response for preventing allergies. Thereby, probiotics diminish allergic inflammation by contributing to provide the balance of increased butyrate, cytokine response, and decreased eosinophil production (Eslami et al., 2020; Lunjani et al., 2020; Royal and Gray, 2020).

Furthermore to the present authors' knowledge novel/natural treatment protocols are necessary, which prompted us to perform the present study. Therefore, our purpose was to establish a natural rectal enema protocol by use of phyto agents and probiotic combination. This protocol was directed to intestinal permeability restorative compounds, which were preferred.

MATERIALS AND METHODS

Study population

A total of 10 dogs with caFr, at the age of 2 to 7 years old, of both sexes, from various breeds.

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Diagnosis-The most challenging part

In the present study in an attempt to take steps for a tentative diagnosis of caFr: i) in vitro serum testing for selected/specific IgE (Polycheck in vitro IgE testing, Biocheck, distributed by RDA Grup, Turkey) ii) intradermal testing of different antigens (Artuvetrin® Skin Test, Nextmune, The Netherlands), iii) elimination diet trial (Bethlehem et al., 2012; Ricci et al., 2013; Mueller and Olivry, 2017) and iv) treatment based respond evaluation. Clinical signs were challenging (alopecia, crusting, scaling etc.). Pruritus scoring deemed available visual analog scale (pVaS).

Food challenge test

The food challenge testing is denoted as improvement within the clinical signs (whether if) based on pVaS ≤ 2, similar to what has been described elsewhere (Shimakura, 2021). This procedure was attended as the first part of this trial and denoted as an active therapeutical intervention, which was followed by the second part of our study 6 weeks after the food challenge test. Even if the challenge was initiated on the first day through animal the owners keep eye on the dogs at natural sources for 3 days. The complete procedure [challenging, consumption of original food, recording the timeline of prior clinical signs detected along with the body locations affected] was very similar to the previous description (Shimakura, 2021).

Ural Breakthrough Nutracuetical Component: pro-active treatment

This treatment second line, however baseline pro-active, protocol involving natural compounds were given in rectal route similar to what has been described elsewhere (Ural et al., 2021a,b). Briefly on 5 different nutraceutical sessions, with 15 minutes apart from each other relevant ones, the necessary combination was prepared (as formula was shown in Figure 1. involving palliative submarine), which was then rectally administered as an enema model (Ural et al., 2021a,b). The treatment trial involved 10 days for rectal enema. Each nutraceutical intervention (shortly abbreviated as Nutr-i1 to Nutr-i5) was performed by the researchers involved at this study with deep experience. Oral consumption of altered calendar probiotic therapy (Ural et al., 2020) lasted at month 3, weekly changed.

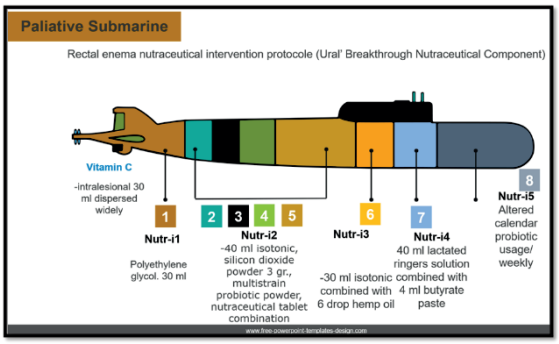

Figure 1. Nutraceutical interventions (Nutr-i) were numbered by their relevant usage order as a rectal enema.

Nutr-i1: Golytely oral sol. powder [polyethylene glycol (pEg 3350)], **Nutr-i2:** Zoosorb Powder (silicon dioxide), Assos Gutfeel Powder (multistrain probiotic), Theravet Skin Tablet [multi polyphenol ingredient nutraceutical formulized by the first author of this research, K.U.], **Nutr-i3:** Theravet Hemp Oil (pure hemp oil formulized by the first author of this research, K.U), **Nutr-i4:** Nucron Paste (Butyrate along with other nutraceuticals), **Nutr-i5:** oral usage of Triogermila I Vial [*Bacillus subtilis*, *Bacillus caogulans*, *Bacillus clausii*] 2x1 p.o. and Twippy Drop 2x10 p.o. [*Lactobacillus rhamnosus* (GG), *Lactobacillus acidophilus*, (LA-5®), *Streptococcus thermophilus* (STY-31™), *Bifidobacterium animalis subsp.Lactis* (BB-12®), *Lactobacillus delbrueckii subsp. Bulgaricus* (LBY-27™)]

Descriptive statistical interpretation

Descriptive statistics for enrolled values were tabulated in an attempt to indicate the mean and standard deviations. Wilcoxon test was used to determine the statistical differences of pruritus Vas scores prior to and thereafter treatment. A p value of < 0.05 was considered significant by using the SPSS 26.0 program (IBM, USA) in all analyzes.

RESULTS

Demographic and evidenced based clinical photographs were deemed available between Figure 2-5 showing 5 out of 10 cases. A mini atlas was shown above in an attempt to attract the interest of readers. Another purpose was to perform a small-scale lesion mapping which will guide readers for their future and similar cases. pVas scores were shown in Table 1 above.

This lesion mapping should have helped audiences for better understanding the mechanism of action and therapeutical outcomes. Furthermore, all cases were experienced at the clinic with special reference to its gut-brain-skin axis. Regarding this axis, clinical findings should be directed to neurotransmitters, neuroendocrine network, and messenger molecules.

Figure 2. Dorsolomber lesions (crusting, total alopecia, hyperpigmentation and comedone formation) were evident, excluding Favrot Criteria and atopic dermatitis. One of the hardest cases, existed at this study, which was very well respond to the Nutr-i1 and -i5 treatment protocol.

Figure 3. Two different cases with caFr. As seen above lesion demography was not very unfamiliar, however some similarities (alopecia, dorso-lomber lesions etc.). Both dogs were responsive to the Nutr-i1 and -i5 treatment protocol used in this study.

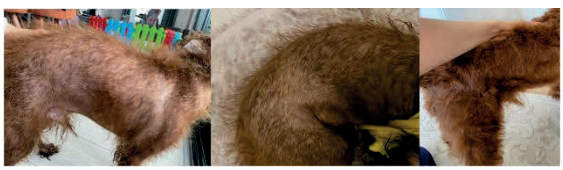

Figure 4. Latero-lateral lesions in a dog with caFr, previously nourished with a high carbonhydrate diet, which was then altered by the present researchers and siwtched to 17% carbonhydrate involving commercial dog food along with rectal enema nutraceuticals.

Figure 5. Resolution and withdrawal of hyperpigmentation and pruritus ani in a dog with caFr which was very good responder to the treatment protocol used in this study.

DISCUSSION AND CONCLUSION

In this part of this manuscript, the researchers will in-depth and separately discuss nutraceutical interventions, for a better understanding of the mechanism of action. Finally will mix relevant data for a conclusion. All 5 and different set of rectal enema would be briefly focused on the mechanism of action within the scope of the present study.

Hemp oil

At inflammatory conditions various cellular pathways might be active within the intestinal environment, directing to a probable pathological condition (Wood et al., 1999; Massa et al., 2004). Human ileum and colon tissues expressed functional CB1 receptors along with elevated populations of CB1-expressing cells following inflammation (Pertwee, 2001; Massa et al., 2004). Considering the small intestine, CB1 receptors involvement for the management of intestinal motility at croton oil-induced inflammation was well recognized. Cannabinoid usage was capable of delaying gastrointestinal transit to those of croton oil-treated mice (Izzo et al., 2001). Furthermore, it was claimed that elevated CB1 receptor expression in jejunum inflammation might bestow within this preservative efficacy. CB1 receptors tempered gastrointestinal motility in the course of croton oil-induced inflammation among mice (Izzo et al., 2001; Nagarkatti et al., 2009). The potential anti-inflammatory efficacy of cannabinoids was discussed in a well-designed review (Nagarkatti et al., 2009), which could attribute to the nutraceutical efficacy obtained at this study herein. The endogenous cannabinoid system is capable of prevention as opposed to inflammatory alterations. All aforementioned facts proposed that activated CB1 and the endogenous cannabinoid system is an untimely and significant physiological pace in colon defense against inflammation (Nagarkatti et al., 2009). All data support that cannabinoids, hemp oil was the choice in this study, adjusting the tissue response as oppose to overwhelming inflammation in the colon (Nagarkatti et al., 2009). Furthermore, given hemp seed oil might mitigate against several dermatitis types [i.e. eczema, seborrheic dermatitis, psoriasis, lichen planus, and acne roseacea] (Tabassum and Hamdani, 2014) and be supportive against bacterial, viral, fungal infections and for the control of scabies (Olsen et al., 2001), available efficacy in the present study should be related to secondary dermatological supportive effects of hemp oil.

Polyethylene glycol

As one of the most multi-faceted molecules, pEg are concomittantly destinate various pathological conditions [immune activation, tight junction function, cell membrane dysfunction, tissue edema and the integrity of the mucosal barrier]. Beacuse of their large molecular volume and hydrophilic chattels, pEg give rise to an oncotic plunge to confiscate water molecules and diminish tissue edema. Mechanism of action for pEG include i) free-radical scavengers, ii) patching damaged cell membranes by formation of reversible complexes with membrane lipids, in an attempt to maintain cell integrity, iii) substitute mucins for preserving and restoring the epithelial mucin layer (Valuckaite et al., 2013), iv) protection of intestinal epithelial cells against various stressors (Valuckaite et al., 2009; Edelstein et al., 2011). In the present study as used in Nutr-i1 formula, pEg could have helped to dampen inflammatory conditions (along with other protocol steps used) and might be responsible for the restoration of the integrity of the mucosal barrier (Ural et al., 2021c).

Silicon dioxide: an enterosorbent

The siliceous enterosorbent silicon dioxide was frequently investigated for its effective heavy metal removal (Li et al., 2011). On the other side it was proposed oral consumption of siliceous small nanoparticles exhibited a risk for worsening intestinal inflammation through activation of the ASC inflammasome (Yazdi et al., 2010; Ogawa et al., 2021). In the present study it was suggested that rectal enema protocol (not oral usage) Nutr-i2 involving silicon dioxide might have helped recovery by toxin/heavy metal binding efficacy (Agaba et al., 2018).

Butyrate

Given major short-chain fatty acids [acetate, propionate, and butyrate], take part in significant roles in the conservation, boosting, and safeguarding of the intestinal tight junction barrier. Among short-chain fatty acids, various mechanisms of action for butyrate on the tight junction barrier has been proposed: i) stimulation of the epithelial metabolism along with depletion of intracellular oxygen, consequently enhancing barrier integrity (Kelly et al., 2015), ii) induction of claudin-3 expression via the Akt pathway in the colon (Yan and Ajuwon, 2017; Feng et al., 2018), iii) elevation of lipoxygenase expression and tight junction barrier integrity through cellular production of hydroxyeicosatetraenoic acid in Caco-2 cells (Ohata et al., 2005). As has been used as Nutr-i4 protocol in this study lactated ringer solution was used as a substrate for commercially available (ready-to-use) butyrate. Utilization of lactate specifically attributed to solely selected bacterial species within clostridial cluster XIVa, though not belonging to all butyrate-producing bacteria (Duncan et al., 2004). In a prior well-written review *Clostridium butyricum* and other relevant microbial communities as dark fermentation bioreactors were denoted as cell factories converting lactate and acetate to butyrate (Detman et al., 2019). Lactate along with butyrate usage should have helped recovery observed at this study. Researcher group of this study with the discovery and guidance of the first author has been elucidating.

Polyphenol combination

Commercially available Nutr-i2 involved a well-designed and formulated (by the first author K.U.) polyphenol combination involving licorice root, pumpkin seed, broccoli sprout, black cumin (*Nigella sativa*), vitamins A-D3-E, zinc-oxide, garlic extract, biotin, collagen and evening primrose which could all contribute to anti-oxidant, anti-inflammatory and anti-histaminic, anti-infectious features of this compound (Theravet Skin Tablet, Naturmed, Antalya, Turkey) used. Dedicated to the first (founder) authors' surname, Ural Breakthrough Nutraceutical Component (Figure 1), this pro-active treatment protocol involved natural compounds given in rectal route similar to what have been described previously (Ural et al., 2021a,b). Briefly on 5 different Nutr-i sessions (as a palliative submarine model), with 15 minutes apart from each other relevant ones (shown in Figure 1), which was then rectally administered as an enema model (Ural et al., 2021a,b). Treatment trial involved 10 days for rectal enema. Each session (from Nutr-i1 to Nutr-i5) was performed by the researchers involved at this study with deep experience. As mentioned above mechanism of action for active ingredients (Table 2) might have helped recovery available at this study.

Table 1. pVaS scores prior to and thereafter Nutr-i1 and -i5 for all cases were deemed available and completely finished study without any side effects.

Score	*Before Treatment* ($\overline{X}$ ± Sd)	*After Treatment* ($\overline{X}$ ± Sd)	*P value*
Vas	7.7 ± 1.62	1.4 ± 1.27	0.005

Vas: Visual analog scale

Probiotic

Two different version of probiotic selection were deemed available: namely soil based one (Triogermila-I Vial, Algae Pharma, İstanbul, Turkey) and Twippy Drop (Valens, İstanbul, Turkey). This previously described altered calendar probiotic therapy (Ural et al., 2020) was lasted at month 3, in which probiotic selections were weekly changed.

Streptococcus thermophilus, which was one of the involved probiotics at Twippy Drop, presented effective support in dermatological disorders both *in-vitro* and *in-vivo* studies; i) elevating the production of beneficial lipids in the stratum corneum [i.e. ceramides, employing moisture in the skin (Di Marzio et al., 1999) and phytosphingosine, combatting *C. acnes* (Pavicic et al., 2007). In this study this probiotic species could have helped restoration of beneficial lipids in the stratum corneum, as reported above.

In the present study 3 different Bacillus strains were composed of soil based probiotic choice for therapeutical armamentarium. Bacillus probiotics possess their benefits by digestive enzyme production (Danilova and Sharipova, 2020). Bioactive probiotic molecules exhibited inflammation combatting efficacy through a) exopolysaccharides, b) molecules of cell envelope c) secretion of proteins. Given prior description of cell envelope-associated probiotic molecules, accompanied by secreted protein and carbohydrate probiotics, exopolysaccharide (ePs) exhibited by *B. subtilis* might have helped combatting against inflammation (Zamora-Pineda et al., 2022). The latter anti-inflammatory action of *B. subtilis* prevented different T cell-mediated diseases and alleviated allergic eosinophilia (Swartzendruber et al., 2019).

In conclusion at the present study altered calendar probiotic therapy all contributed to recovery obtained in all cases, as brief explanation was given herein. All Nutr-i1 to Nutr-i5 protocol showed pro-active treatment efficacy, which could contribute to novel protocol establishment.

Table 2. Selected active ingredients of Nutr-i2 and mechanism of action by references.

Active Ingredients	Mechanism of action
Licorice root (root extract)	-Skin protection for combatting oxidative stress (Castangia et al., 2015; Mostafa et al., 2014) -Acceleration of wound epithelization Kotian et al., 2018) - Efficacous for diminshing atopic dermatitis (Yu et al., 2017) -Acive component, glabridin, acts as antioxidant, estrogenic, anti-inflammatory, and skin-whitening agent (Simmler et al., 2013), exhibits skin depigmentation activity (Pastorino et al., 2018).
Pumpkin seed	-Anti-inflammatory against facial acne (Al-Noor, 2017) -Block the action of 5-alpha reductase, antiandrogenic effects (Esfandiari and Kelly, 2005; Kwon et al., 2007)
Broccoli sprout (Brassica oleracea L. var. italica Plenck)	-Antioxidant (Fahey and Talalay, 1999). -Antiinflammatoric (Talalay, 2007) and -Antimutagenic agents (Kern et al., 2007)
Black cumin (*Nigella sativa*)	-Anti-viral (Ma et al., 1994; Salem and Hossain, 2000) -Anti-fungal (Kader et al., 1995; Aljabre et al., 2005) -Effective agaisnt vitiligo (Ali and Meitei, 2011)

Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept: K.U., Design: K.U., Data Collection or Processing: K.U., H.E., S.E., T.A., C.B., G.G., Analysis or Interpretation: K.U., H.E., Literature Search: K.U., Writing: K.U., H.E.

Financial Support

This research received no grant from any funding agency/sector.

Ethical Approval

This study was obtained from routinely ill dogs admitted to the Internal Medicine Clinic of the Faculty of Veterinary Medicine, Adnan Menderes University, and no procedures were performed in dogs for study purposes, and the diagnosis and treatment intervention were obtained within the scope of routine clinical evaluation. This study was performed according to The Declaration of Helsinki, Ethical Principles.

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Investigation of Dilated Cardiomyopathy Tendency by Echocardiography in Kangal Dogs

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Abstract

Dilated cardiomyopathy (DCM) is defined as a cardiac disease characterized by dilatation of the cardiac chamber, decreased myocardial contractility, and decreased left ventricular ejection fraction. In this study, we aimed to determine whether there is a tendency to age-related dilated cardiomyopathy in Kangal Dogs, a breed of Anatolia, echocardiographically. The experimental groups of the study consisted of a total of 20 dogs, 10 of which were 1-3 years old, young group (Group I) and the other 10 were 4-7 years old, middle-aged group (Group II), who had no clinical problems at rest. By echocardiographic examination, the end-diastolic thickness of interventricular septum, the end-systolic thickness of interventricular septum, left atrial diastolic diameter, aortic root diastolic inner diameter, the ratio of left atrium diameter to aortic diameter, left ventricular end-diastolic inner diameter, left ventricular end-systolic inner diameter end-systolic thickness of the posterior wall, the end-diastolic thickness of the posterior wall of the left ventricle, heart rate, left ventricular ejection fraction, left ventricular contraction force, stroke volume, cardiac output, end-diastolic volume and end-systolic volume parameters were measured. The data collected from the two groups were analysed using independent samples T-test. In conclusion, considering that the age of the Kangal dogs constituting the middle-aged group (G II) of the study was within the age limits specified in the studies (6-7 years), a myocardial defect indicating echocardiographic DCM was not detected. Thus, it was concluded that there is no tendency for DCM among the age groups included in the study. However, it can be said that echocardiographic evaluation of more coiled dogs in the given age group may give a more reliable result considering that the rate of DCM is 6% in different breed dogs.

Keywords: Dilated cardiomyopathy, tendency, Kangal, Dog.

INTRODUCTION

Cardiac muscle disorders are referred to as cardiomyopathies (CMPs). CMPs lead to cardiac dysfunction resulting in decreased strength of heart muscle contraction (systolic failure) or decreased myocardial relaxation (diastolic failure). Apart from these, damaged heart muscle cells generate impulses as an ectopic focus and cause supraventricular or ventricular arrhythmias (O'Grady and Sullivan, 2004; Gökçe, 2014; Colakoglu and Sahal 2015; Turgut, 2017).

CMPs can emanate primarily or secondarily. The primary group of CMPs includes; dilated, hypertrophic, restrictive and intermediate cardiomyopathies (O'Grady et al., 2008; Gökçe, 2014). In the secondary group of CMPs, there are; toxic, inflammatory, nutritional, metabolic, infectious and infiltrative cardiomyopathies (Mansilla et al., 2019).

Dilated cardiomyopathy (DCM) is characterized by dilatation of the cardiac chamber, decreased myocardial contractility, and decreased left ventricular ejection fraction (Bonagura and Lehmkuhl, 2000). Primarily, dilated cardiomyopathies are known to be a vital cause of heart failure in large dog breeds. DCM is a disorder that progresses insidiously, reduces the contractility of the heart and eventually, results in congestive heart failure (Colakoglu et al., 2022). Heart failures due to secondary and infectious causes are seldomly seen (Grady and Sullivan, 2004; Turgut, 2017).

Although etiologically, primary DCM in dogs may occur due to genetic, familial, age-related, hormonal (hypothyroidism) and metabolic (carnitine, taurine, selenium deficiency) causes, it is mostly expressed as idiopathic DCM (Ettinger and Feldman, 1995; Bonagura and Lehmkuhl, 2000; Turgut, 2017). It is stated that genetic and familial factors play an essential role in most DCM cases and breeds such as; Doberman Pinscher, Scottish Deerhound, Irish Wolfhound, Great Dane, Saint Bernard, Afghan Hound, Newfoundland, Old English Sheepdog, Cocker Spaniels are included (Osterziel et al., 2005; O'Grady et al., 2008; Wiersma et al., 2008; Stephenson et al., 2012; Turgut, 2017). This disease is more common in middle (4-6 years old) and older dogs (Onmaz et al., 2011; Gökçe, 2014). In addition, it is emphasized that factors affecting the functions of myocytes, myocarditis with myocyte necrosis (parvovirus infections), global myocardial ischemia, and toxic destruction of myocytes (doxorubicin and monensin) may also cause dilated cardiomyopathy secondarily (Bonagura and Lehmkuhl, 2000).

DCM develops insidiously in three phases. In the first phase, the heart is morphologically and electrically normal and there is an asymptomatic picture. In the second phase, there is morphological cardiac enlargement and/or electrically ventricular ectopia. However, there is no clinically striking finding (latent course). The third phase is the period in which congestive heart failure (CHF) develops with clinical symptoms (O'Grady et al., 2008).

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Regarding the pathogenesis of the disease, two prominent histopathological disorders are known (Turgut, 2017). First and foremost, myocardial cells are deteriorated giving them a thin-wavy appearance during the latent period, that is, in the preclinical period, which is also considered as a predisposition to DCM (Wiersma, 2008). Secondly there are pathological disorders characterized by myocyte atrophy, lysis, fat infiltration and fibrosis in cardiac muscle cells. In the preclinical stage, dilatation begins to form in the heart cavities as the continuation of the cardiac muscle disorders. Parallel to this, while cardiac index (CI) decreases, sympathetic, hormonal and renal compensatory mechanisms are activated, causing an increase in heart rate, peripheral vascular resistance, and volume retention (Gökçe, 2014; Turgut, 2017). Systolic function deteriorates progressively and all cardiac chambers dilate and the diameter of the cardiac chamber increases, most notably in the left ventricle and left atrium. With the progression of dilatation, the ratio of left ventricular wall thickness to lumen volume decreases. This leads to decreased systolic function, dysfunction in papillary muscles, and regulations due to atrioventricular valve insufficiency (Bonagura and Lehmkuhl, 2000).

As a result of the progressive decrease in systolic function, an increase in left atrial and left ventricular pressure occurs at the end of diastole. Therefore, diastolic insufficiency develops in the dilated ventricle. Owing to that, loss of function in both ventricles and all the symptoms of congestive heart failure (CHF) manifest (Bonagura and Lehmkuhl, 2000; Gökçe, 2014, Turgut, 2017). Acute and severe ventricular arrhythmias may develop in such sick dogs and ultimately, may result in sudden death.

Patients in the latent period and the preclinical stage are asymptomatic (Bonagura and Lehmkuhl, 2000; O'Grady et al., 2008; Turgut, 2017). With the onset of left ventricular dysfunction, tachyarrhythmia emerges as an important symptom. In symptomatic dogs, cardiac arrhythmia, exercise intolerance, laziness, and fatigue predominate as signs of CHF. Aside from that, right and/or left CHF symptoms are seen (O'Grady et al., 2008; Turgut, 2017).

DCM can be diagnosed by evaluating radiographic, echocardiographic, electrocardiographic and laboratory findings (Bonagura, 1994; Dukes-McEwan et al., 2003; Wess et al., 2010; Stephenson et al., 2012; Turgut, 2017). Vollmar (1999), stated that Echocardiography (ECHO) is an important diagnostic method in the evaluation of heart diseases and that it can be used as an effective method in the early diagnosis of DCM in the preclinical stage. Hence, echocardiography is the "gold standard" method used in the diagnosis of DCM (Bonagura and Lehmkuhl, 2000; Wess et al., 2010; Jeyaraja et al., 2015). Also, the European Society of Veterinary Cardiology (ESVC) recommended that the diagnosis of DCM should be based on the results of 2D and M-mode echocardiographic examinations (Dukes-McEwan et al., 2003). For echocardiographic confirmation of DCM, left ventricular dilatation, decreased systolic myocardial function, and increased left ventricular sphericity should be detected (Dukes-McEwan et al., 2003). Generally, these disorders in the left heart are accompanied by right heart dilatation (Bonagura and Lehmkuhl, 2000).

With echocardiographic examination, cardiac functions can be interpreted based on quantitative values such as; fractional contraction of the heart muscle, ejection fraction, atrial and ventricular cavity dimensions, and qualitative evaluations such as; hypokinesia and hyperkinesia (Ettinger, 1991).

In this study, it was aimed to investigate whether DCM, which is progressive heart disease, causes CHF in patients in a short time, and causes death by affecting mostly large dog breeds, has a tendency to develop age-relatedly in Kangal dogs.

MATERIALS AND METHODS

The experimental groups of the study consisted of a total of 20 dogs, 10 female and 10 male Kangal dogs, aged between 1-7, which were domesticated by indigenes, did not have any clinical conditions at the time of rest. These dogs were divided into two groups, 10 (5 females, 5 males) 1-3 years old, young group (Group I=G1), 10 (5 females, 5 males) 4-7 years old, middle-aged groups (Group II).

Before the ECHO examination of the dogs used in the study, pulse and respiratory frequency, body temperature and mucosal examinations were performed. Then, in a calm and stress-free environment, M-mode echocardiographic examinations for DCM were performed from the right parasternal region with a Z6-Vet echocardiography device following the technique's protocols (Bonagura et al., 1985; Dukes-McEwan et al., 2003; Wess et al., 2010; Stephenson et al., 2012; Jeyaraj et al., 2015). The right parasternal window shaved with razor.

The right parasternal window was used for measurements on the left ventricle. Measurements were performed on the frozen M-mode image using the M-mode cursor under the guidance of 2-D echocardiography on the long axis from the right parasternal window (Lombard, 1984; Sahn et al., 1978).

In the case of diastole of the heart, all measurements on the long axis were made when the anterior movement of the posterior wall of the left ventricle was in the posterior position, and in the case of systole, all measurements were made when the anterior movement of the posterior wall of the left ventricle was in the most anterior position (Kayar, 2001).

First, in the echocardiographic examination, the heart of all dogs was subjectively evaluated on the right parasternal long axis four and five chamber view (RPS LAx-4C and RPS LAx-5C, respectively). Secondly, the right parasternal short axis (RPS SAx) papillary muscle level view was used for measuring the end-diastolic thickness of the interventricular septum (IVSd), the end-systolic thickness of the interventricular septum (IVSs), left ventricular end-diastolic inside diameter (LVIDd), left ventricle end-systolic inside diameter (LVIDs), the end-systolic thickness of the left ventricular posterior wall (LVPWs) and the end-diastolic thickness of the left ventricular posterior wall (LVPWd); RPS SAx aortic valve level was used for measuring the left atrial diastolic diameter (LA), the diastolic inner diameter of the aortic root (Ao) and the ratio of the left atrium diameter to the aortic diameter (LA/ Ao). Also, for measuring mitral valve leaflets, RPS Sax mitral valve level was used. Moreover, heart rate (HR), left ventricular ejection fraction (%EF), LV fractional shortening (%FS), stroke volume (SV), cardiac output (CO), the end-diastolic volume (EDV) and the end-systolic volume (ESV) parameters were determined.

IVSd is the distance between the endocardium facing the left ventricular cavity of the interventricular septum at the end of diastole and the endocardium facing the right ventricular cavity, and IVSs is the distance between the

interventricular septum facing the left ventricular cavity and the endocardium facing the left ventricular cavity at the end of systole (Boon, 1998).

LA was measured as the distance from the outer end of the posterior aortic wall to the endocardial border of the left atrial wall at the end of diastole, and Ao was measured as the distance from the anterior wall of the aorta to the posterior wall (Lombard and Spencer 1985; Vollmar 1999).

The ratio of the diameter of the left atrium to the diameter of the aorta (LA/AO) was calculated by dividing the diameter of the left atrium by the diameter of the aorta (Lombard and Spencer, 1985; Boon, 1998).

LVIDd is the distance from the lower point of the intraventricular septum at the level of the chorda tedinea at the end of diastole to the upper point of the posterior wall of the left ventricle, and LVIDs is from the lower point of the upper intraventricular septum posterior wall of the left ventricle at the end of systole. Measured as the distance to the point (Kittenson et al., 1984; Gooding et al., 1986; Haggostrom et al., 1996).

LVPWd was measured as the thickness from the inside of the ventricular posterior wall to the intra-wall pericardium at the end of diastole, and LVPWs was measured as the thickness from the inside of the ventricular posterior wall to the intra-wall pericardium at the end of systole (Boon, 1998; Kayar, 2001).

Heart rate (HR) was measured as the distance between two systoles in the direction of the left ventricular posterior wall facing the left ventricular cavity (Boon, 1998).

Using the values obtained from the left ventricular Ejection Fraction (EF %), EF % was calculated with the formula; EF % = $[(LVIDd)^3 – (LVIDs)^3] /(LVIDd)^3$ x 100 (Koch et al., 1996).

Left ventricular Fractional Shortening (FS%), using the obtained values, was calculated with the formula FS% = [(LVIDd–LVIDs) / LVIDd] x 100 (Bayon et al., 1994).

Using the Teicholz method, stroke volume (SV) is; flow velocity x cross-sectional area. Cardiac output (CO) is the volume of blood ejected from each ventricle per unit time and is calculated with the formula; HR x SV. Body surface area (BSA) was used to calculate the cardiac index. Cardiac index (CI) was calculated with the formula; CO/BSA. End-diastolic volume (EDV) and end-systolic volume (ESV) parameters were also determined by the Teicholz method (Turgut, 2017).

The obtained data were analysed using independent samples T-test to determine the difference and significance between the groups at $p<0.05$ significance level (Akgül, 2005).

RESULTS

The clinical findings and echocardiographic measurement results of the Kangal dogs, which formed the animal material of the study and were divided into two groups (Group I; young group 1-3 years old, Group II; middle-aged group 4-7 years old) according to age ranges, are summarized in Table 1 and 2. In addition, some of the echocardiographic images obtained are presented in figures 1, 2 and 3.

Table 1. Clinical examination results of the dogs used in the study.

Parameter	Group I (n=10)	Group II (n=10)
	Mean ± Standard Deviation	Mean ± Standard Deviation
Pulse Frequency (number of beats /minute)	90.90 ± 16.99	83.44 ± 13.41
Breathing Frequency (r/minute)	20	16
Body Temperature (^{0}C)	38.7	38.5
Capillary Refill Time (seconds)	2	2
Mucous Membranes (rose pink)	+	+
Body Weight (kg)	49.50 ± 6.43	59.37 ± 3.20

Table 2. Echocardiographic findings of young and middle-aged groups.

Parameter	Group 1 (n=10)	Group 2 (n=10)	P
	Mean ± Standard Deviation	Mean ± Standard Deviation	value*
LA (cm)	3.27 ± 0.31	4.07 ± 1.23	0.06
AO (cm)	3.00 ± 0.28	3.29 ± 0.20	0.01
LA/AO	1.10 ± 0.16	1.22 ± 0.31	0.27
IVSd (cm)	1.06 ± 0.11	1.04 ± 0.06	0.64
LVPWd (cm)	1.15 ± 0.17	1.24 ± 0.18	0.27
LVIDd (cm)	4.59 ± 0.50	5.17 ± 1.06	0.14
IVSs (cm)	1.296 ± 0.20	1.30 ± 0.14	0.94
LVPWs (cm)	1.48 ± 0.31	1.48 ± 0.12	0.97
LVIDs (cm)	3.09 ± 0.46	3.54 ± 1.11	0.25
HR (Beat /minute)	90.90 ± 16.99	83.44 ± 13.41	0.30
ESV (ml)	39.52 ± 16.11	60.44 ± 56.20	0.27
EDV (ml)	98.26 ± 25.13	133.26 ± 78.64	0.19
EF (%)	59.93 ± 8.22	57.06 ± 10.43	0.50
SV (ml)	58.73 ± 15.58	72.96 ± 24.13	0.13
CO (L/minute)	5.43 ± 1.97	5.49 ± 1.66	0.94
SI (ml/m^2)	38.52 ± 10.41	38.78 ± 6.84	0.95
FS (%)	32.36 ± 5.89	31.11 ± 6.85	0.66
CI (ml/min/m^2)	3.59 ± 1.42	3.14 ± 1.17	0.48
Body Weight (BW) (kg)	49.50 ± 6.43	59.37 ± 3.20	0.001
Body Surface Area (BSA) (m^2)	1.53 ± 0.15	1.72 ± 0.64	0.001

* P<0,05: Level of Significance

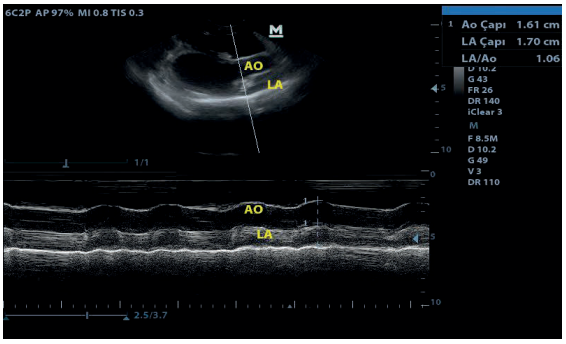

Figure 1. M-mode imaging of the left atrium and aorta.

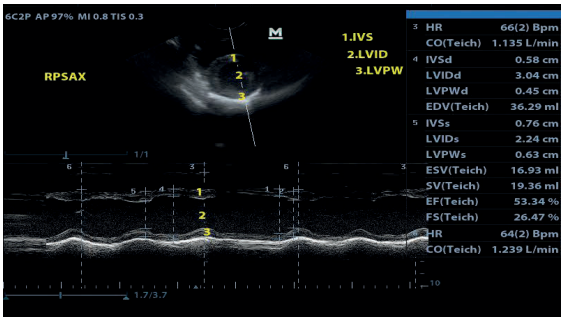

Figure 2. Measured image of intraventricular septum thickness, left ventricular diastolic inner diameter and left ventricular posterior wall thickness.

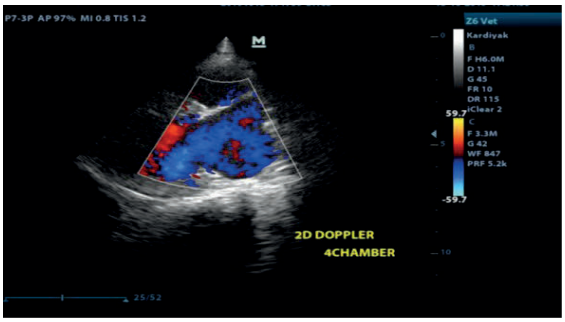

Figure 3. Right Parasternal Long axis 4 Chamber Color Doppler image (normal blood flow).

From the results of the study, although there were differences between groups in left atrium diastolic diameter (LAd), left atrium diameter to aorta diameter ratio (LAd/AOd), the diastolic thickness of the interventricular septum (IVSd), left ventricular end-diastolic posterior wall thickness (LVPWd), left ventricular end-diastolic inner diameter (LVIDd), the end-systolic thickness of the interventricular septum (IVSs), left ventricular end-systolic posterior wall thickness (LVPWs), left ventricular end-systolic inner diameter (LVIDs), pulse frequency (HR), end-systolic volume (ESV), end-diastolic volume (EDV) ejection fraction (%EF), stroke volume (SV), cardiac efficiency, cardiac output (CO), stroke volume index (SI), left ventricular facial contraction force (%FS), and cardiac index (CI) measurements, these differences were not statistically significant (Table 2).

When the mean AOd value of the young group (G I) and that of the old group (G II) was compared, that of G II (3.29 ± 0.20) was higher than G I (3.00 ± 0.28) and was found to be significant at $p< 0.01$.

It was determined that the mean body weights (BW) of the young group (G I) among the Kangal dogs included in the study (49.50 ± 6.43) were lower than those of the middle-aged group (G II), and this was statistically significant at $p<0.01$.

It was found that the mean body surface area (BSA) (1.53 ± 0.159) of the young group (G I) was lower than the mean body surface area (1.72 ± 0.64) of the middle-aged group (G II) and this was statistically significant at $p<0.01$.

An EF value below 55% was accepted as the limit for systolic dysfunction. While systolic dysfunction was detected in 2 dogs (20%) in GROUP 1, systolic dysfunction was observed in 4 dogs (40%) in group 2. One of the dogs in Group 2 with systolic dysfunction also had a high LA/Ao ratio (LA/Ao: 1,93).

DISCUSSION AND CONCLUSION

Primarily formed dilated cardiomyopathies (DCM) are an important cause of heart failure in large dog breeds and cause dilatation of the cardiac chamber, decrease in myocardial contractility and decrease in left ventricular ejection fraction (Bonagura and Lehmkuhl, 2000; O'Grady and Sullivan, 2004; Jeyaraja et al., 2015; Turgut, 2017; Colakoglu et al. 2022).

In our study, we aimed to determine whether there is a tendency to age-related dilated cardiomyopathy in 1-3 and 4-7 years old Kangal Dogs, a breed unique to our country, Turkey, by using 2-D and M-mode echocardiographic techniques.

O'Grady et al., (2008) state that the pre-clinical phase of DCM has a latent period, and at the end of the progressive state, congestive heart failure (CHF) develops with clinical symptoms. It was observed that there was no clinical finding indicating DCM in the Kangal dogs included in the study (Table 2). While no significant finding was found in this phase even with echocardiography, it is stated that during this phase only myocardial cells deteriorate and this can be revealed by histopathological examination (Wiersma et al., 2008).

It has been reported that a decrease in arterial blood pressure, deterioration in pulse quality, arrhythmia, prolongation of capillary filling time and pallor of the mucous membranes may develop in the phases after the preclinical, clinical phase and in very advanced cases of CHF (Bonagura and Lehmkuhl, 2000; Wess et all., 2017). However, none of the pathological findings mentioned was encountered in our study.

In the study conducted, in the examination of the ejection fraction values of the young (G I) and middle-aged groups (G II), it was found that the mean G I value was higher than the mean value of the G II, but this difference was not statistically significant ($p=0.50$) (Table 2). These values are consistent with the values obtained by Kayar, (2001) from the healthy Kangal dogs and the values obtained by Jeyaraja et al., (2015) from the healthy control, Labrador retriever dogs.

Some researchers state that left atrium diastolic diameter and aorta diastolic diameter are positively correlated with body weight and body surface area, and may increase physiologically with age (Lombard, 1984; Vollmar, 1999). In our study, the body weight, body surface area, and aorta inner diameter values of the middle-aged group were found to be higher than the values of the younger group (Table 2). However, since the LAd/AOd ratios in both groups were within normal physiological

limits, the above difference was not considered as a finding for DCM.

Increased left ventricular end-diastolic inside diameter (LVIDd) and left ventricular end-systolic inside diameter (LVIDs) were found in some echocardiographic studies in dogs with DCM (Vollmar, 1999; Stephenson et al., 2012; Jeyaraja et al., 2015). However, in our study, left ventricular end-diastolic inside diameter (LVIDd) and end-systolic inside diameter (LVIDs) values and IVSs values were close to each other in the two groups (GI = 1.296 ± 0.20; GII = 1.30 ± 0.14). Similarly, IVSd were found to be close to each other (GI = 1.06 ± 0.11; G II = 1.04 ± 0.06) and there was no statistical difference.

Although in some studies, low % FS, increased LA/AO ratio, increased ESV and EDV values were observed in dogs with DCM. In our study, despite the individual differences in the parameters of both groups, there was no statistically significant difference between the groups (FS% – p=0.66; LAD/AOD – p=0.27; ESV-p=0.27; EDV – p=0.19) (Stephenson et al., 2012; Jeyaraja et al., 2015, Vollmar, 1999). Nonetheless, our results were found to be consistent with the findings of Kayar's (2001) study on healthy Kangal dogs (Table 2).

Bonagura and Lehmkuhl (2000), determined that with the progressive deterioration of cardiac muscle functions in dogs with DCM, all cardiac chambers, especially the left ventricle and left atrium, will become dilated. In this case, he states that the diameter of the heart circle will increase and the ratio of left ventricular wall thickness to lumen volume will decrease (wall stress) with the progression of dilatation. It was observed that the left ventricle diastolic and end-systolic wall thickness (LVPWd and LVPWs) values obtained in our study are within physiological limits as stated by Kayar, (2001) and there is no condition indicating wall stress in both GI and G II.

After the preclinical stage, during the progression of the cardiac muscle disorders, dilatation begins to form in the heart cavities and parallel, the cardiac index (CI) decreases (Gökçe, 2014; Turgut, 2017). When the cardiac index values of both groups obtained in the study were examined, the mean values of G I (3.59 ± 1.42) were higher than those of G II (3.14 ± 1.17) but statistically insignificant (p = 0.48) (Table 2). Two dogs in Group I had systolic dysfunction, compared to 4 in Group II. All dogs having systolic dysfunction had no CHF signs. It can be thought that this situation must be because of preclinic DCM (O'Grady et al. 2008; Colakoglu and Sahal, 2015).

It is known that the evaluation of left ventricular systolic functions provides information about DCM and thus, parameters such as pulse frequency (HR), stroke volume (SV), cardiac output (CO) are important (Turgut, 2017; Wess, 2021). In our study, the mean HR values measured by echocardiography were G I = 90.90 ± 16.99; G II 83.44 ± 13.41; p=0.30, mean SV values GI = 58.73 ± 15.58; G II 72.96 ± 24.13; p=0.13. These results indicate that no statistical difference was found between the groups. Naturally, there was no statistical difference between the groups (GI = 5.43 ± 1.97; G II = 5.49 ± 1.66) in the mean cardiac output (HR x SV = CO) values calculated with these two values (p=0.94) (Table 2).

The results obtained from the examination of the above parameters determined by echocardiography, it was observed that there was no finding indicating the tendency to DCM developing in the 1-3 years old Kangal dogs, which made up the young group (G I) and also, in the 4-7 years old Kangal dogs, which made up the middle-aged group (G II). However, Jeyaraja et al. (2015) reported that the mean age exposing Labrador retriever dogs to DCM was 6.68 ± 0.49 years. Also, Tidholm et al., (1997) found the mean age of 189 dogs with DCM to be 6.6, while Tidholm and Jonsson, (1996) found the mean age of Newfoundland dogs with DCM to be 5 years, and they stated that DCM was age-related. Moreover, in a study conducted by Wess et al., (2010) on Doberman pinchers, DCM was found to be 13,2% in dogs younger than 4 years of age and 44.1% in dogs older than 8 years of age. The author suggested that the probability of DCM in Doberman pinchers increases with age and that dogs should be checked for DCM annually from the age of 2 years. Similarly, Matli et al., (2021) reported that age indicates poor prognosis in dogs over 8 years of age.

In conclusion, considering that the age of the Kangal dogs which constituted the middle-aged group (G II) of the study were within the age limits stated in the studies above, no myocardiological disorder indicating DCM was detected by echocardiography. In other words, the conclusion that there was no age-related tendency to DCM in the Kangal breed dogs can be reached. However, considering the DCM rate of 6% in the study of Jeyaraja et al., (2015) in the Labrador retriever dogs, it can be said that the echocardiographic evaluation of a larger number of Kangal dogs in the specified age group may yield a more reliable result.

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Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept: G.T., S.Y.D., Design: G.T., S.Y.D., Data Collection or Processing: G.T., S.Y.D., Analysis or Interpretation: G.T., S.Y.D., Literature Search: G.T., S.Y.D., Writing: G.T., S.Y.D.

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Ethical Approval

All methods and procedures used in this study comply with the guidelines of the Turkey and EU directive (Directive 2010/63/EU) on the protection of animals used for scientific purposes. This study did not require approval from the authorities or the ethics committee of the institution. However, patient owners were informed and consent was obtained. This study was performed according to The Declaration of Helsinki, Ethical Principles.

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RESEARCH ARTICLE

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Evaluation of the Effects of Medetomidine and Dexmedetomidine Use on Intraocular Pressure in Cats

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Abstract

In the present study, it was aimed to investigate the effects of α-2 agonists used for sedation in cats, namely medetomidine hydrochloride and dexmedetomidine, on intraocular pressure. Two study groups, each containing 17 animals, were formed. One of the groups was treated with 80 µg/kg medetomidine hydrochloride, and the other group with 40 µg/kg dexmedetomidine, via IM route. The animals underwent intraocular pressure measurement before sedative agent administration (T), at the time of vomiting (T1), after vomiting (T2), at 40th minute (T3), at 60th minute (T4), and 20 minutes after atipamezol administration (T5). There was no significant difference between intra-group intraocular pressure measurements taken at different times and inter-group IOP mesaurements taken at the same time points ($P>0.05$). A numerical increase occurred in intraocular pressure after vomiting, and intraocular pressure was found to be lower during sedation than at the baseline. In conclusion, it was determined that the administration of medetomidine hydrochloride and dexmedetomidine via intramuscular route for sedation reduced intraocular pressure but the levels remained within reference range.

Keywords: Anaesthesia, cat, intraocular pressure, tonometry.

INTRODUCTION

Intraocular pressure (IOP) measurement is an important diagnostic test because IOP levels are an important marker of ocular health and disease state. Intraocular pressure has a critical role on the road to success in the treatment and surgery of ophtalmological disorders (Broadwater et al., 2008; Gross and Pablo, 2015). A severe increase in intraocular pressure can damage the optic nerve or increase morbidity and worsen prognosis by causing the prolapse of ocular content in case of corneal and scleral instability. In patients undergoing corneal or intraocular surgery, excitement, coordination disorder, coughing, or gagging are undesired states in the early postoperative period. Therefore, it is desirable to achieve a balanced and multimodal anesthesia with agents that are capable of providing an unproblematic anesthetic recovery (Gross and Pablo, 2015; Bellini et al., 2017).

If sedation is needed for ocular examination or a more detailed ocular evaluation in animals, it is important to consider the effects of pharmacological agents. In veterinary medicine, most of the studies on the effects of different sedative/analgesic and anesthetic protocols on pupil diameter (PD) and IOP have been performed in dogs (Douet et al., 2018; Micieli et al., 2018). It is known that there is a limited number of studies examining the effects of sedatives and analgesics on intraocular pressure in cats (Malmasi and Ghaffari, 2015; Schroder et al., 2018). It is reported that the intraocular pressure is between 9 mmHg and 31 mmHg in a healthy eye in cats (Miller et al., 1991).

α-2 adrenoreceptor agonists are widely used as sedative agents, and are strong tranquilizer and analgesic drugs. Antagonizing their actions with atipamezol provides an advantage for their use. Medetomidine is the most commonly used α-2 adrenoreceptor agonist in small animals. It causes dose-dependent sedative effect 15-20 minutes after its intramuscular (IM) injection (Murrel JC, 2007). Dexmedetomidine is a α-2 adrenoreceptor agonist and a medetomidine isomer that is very similar to medetomidine with respect to pharmacokinetic properties. Previous studies have reported that α-2 adrenoreceptor agonists possess some ocular side effects (Aghababaei et al., 2021).

This study aimed to investigate the effects of the administration of medetomidine hydrochloride and dexmedetomidine via intramuscular route for anesthesia on IOP in cats, and to antagonize its effects with atipamezol.

MATERIALS AND METHODS

This study was approved by Kırıkkale University Animal Research Ethics Committee (AREC), and the owners of the animals were informed about the study. The study material consisted of 34 cats with healthy eyes, which required sedation for various interventions and examinations (mouth examination, radiological examination, bandage change). Animals that were found unsuitable for sedation with α-2 adrenoreceptor agonists due to the results of complete blood count and biochemical tests were excluded. The cats included in the study protocol were divided into two groups each

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containing 17 animals. One of the groups was administered 80 µg/kg medetomidine hydrochloride and the other 40 µg/kg dexmedetomidine via IM route. The animals underwent intraocular pressure measurement before the administration of the sedative agent (T0), at the time of vomiting (T1), after vomiting (T2), at 40th (T3) minute, and at 60th (T4) minute. Atipamezol was administered 60 minutes later, and the measurement was repeated 20 minutes after atipemazol (T5). Each measurement was repeated for three times, and the average level was recorded if there was no more than 5% difference between the measurements. All measurements were performed using the Tono-Pen Vet® aplanation tonometry device (Reichert, USA). The aplanation tonometry was calibrated before the measurements of each cat.

Statistical Analysis

SPSS v15 (SPSS Inc. Chicago, Illinois, America) statistical software package was used for the statistical analyses of the study data. The study data were tested for normality using the Shapiro–Wilk test and the results showed that they did not meet the parametric test assumptions. Therefore, a nonparametric test was used. Friedman test was used to analyze time-dependent differences within the groups, and Kruskal-Wallis nonparametric test to analyze the difference between the groups. $P<0.05$ was considered statistically significant.

RESULTS

The age of the animals included in the study was 17.1±1.78 months, and their body weight was 3.79±0.96 kg (Mean ± SEM).

There was no significant difference between intra-group intraocular pressure measurements taken at different times and inter-group IOP mesaurements taken at the same time points ($P>0.05$) (Table 1). It was noticed that there occurred a numerical, albeit statistically non-significant, decrease in IOP levels after the administration of α-2 adrenoreceptor agonists, and that those levels were higher after atipamezol injection than those at baseline and during sedation. While some animals were noted to be fully sedated after the injections, some others completed the period with very mild sedation. Those animals also had lower IOP levels during sedation than those at baseline. Twenty-five animals showed an elevation in IOP level after vomiting whereas no IOP change was observed in 9 animals.

Table 1. Intraocular pressure levels of cats sedated with medetomidine hydrochloride and dexmedetomidine before sedative agent injection (T0), after vomiting induced by sedative agent injection (T1), at 20th (T2), 40th (T3), 60th (T4) minutes, and at 20 minutes after atipamezol administration (T5).

	T0	T1	T2	T3	T4	T5
Medetomidine	15.71 ± 2.36	16.12 ± 2.95	15.18 ± 2.43	15.12 ± 2.26	14.76 ± 2.06	16.29 ± 2.41
Dexmedetomidine	15.76 ± 2.01	16.53 ± 3.04	15.41 ± 2.98	15.82 ± 2.92	15.71 ± 2.68	16.82 ± 2.53

DISCUSSION AND CONCLUSION

It is known that α-2 adrenoreceptor agonists cause vomiting, bradycardia, hypotension, hypertension, and IOP changes after their use (Kanda et al., 2005). In general, α-2 adrenoreceptor agonists depress sympathetic tone and may reduce IOP by decreasing humor aqueous production. Studies on small animals have reported that α-2 adrenoreceptor agonists cause a significant decrease in IOP in dogs, while they show no effect in cats at all (Artigas et al., 2012; Micieli et al., 2018). α-2 adrenoreceptor agonists were also used in the present study, and caused no significant change compared with both the baseline IOP level and the reference intraocular pressure levels, and caused only a numerical decrease compared with the baseline level. This indicates that the use of α-2 adrenoreceptor agonists had no unfavorable effects on intraocular pressure in cats.

Reference intraocular pressure measurements made with aplanation tonometry are between 12 mmHg and 32 mmHg in healthy animals (Miller et al., 1991). It is reported that an animal should be followed up for glaucoma and treated as necessary when intraocular pressure exceeds 25 mmHg in dogs and 27 mmHg in cats (Miller, 2008). The present study also showed that the baseline and post-injection levels were within the reference range. This finding was considered to be related to the fact that the sedative agents used did not have an effect on intraocular pressure.

Vomiting is an act that increases intraocular pressure; it particularly worsens deep corneal ulcers, descemetocele, staphyloma, and glaucoma (Rauser et al., 2012; Kanda et al., 2015). This is an important side effect that should be taken into consideration when administering α-2 adrenoreceptor agonists. Although the novel α-2 adrenoreceptor agonists have less vomitory effect, studies have shown that the rate of vomiting in cats is around 20% (Lemke, 2004). A former study used α-2 adrenoreceptor agonists and investigated the effects of nausea and vomiting on IOP. The results of that study showed no positive correlation between vomiting and IOP, and the authors suggested that vomiting do not increase IOP or can increase it for a short time (Wolfran et al., 2022). In the present study, IOP was not measured at the time of vomiting but within 1-2 minutes after vomiting. Although there was no significant difference between the measurements, a small increase was evident after vomiting compared with the baseline level. This may suggest that vomiting can increase IOP.

Wolfran et al., (2022) reported that the use of 7.5 µg/kg dexmedetomidine had no effect on IOP while its use at a dose of 10 µg/kg significantly reduced IOP in cats. Malmasi and Ghaffari, (2015) reported that 100 µg/kg medetomidine caused no effect on IOP in cats. Kanda et al., (2019) reported that medetomidine hydrochloride used at a dose of 80 µg/kg significantly reduced tear production in cats. The present study used 40 µg/kg dexmedetomidine and 80 µg/kg medetomidine; although a very small numerical decrease occurred from the baseline level, this change was statistically non-significant; additionally, all levels were within the

reference range. Regarding medetomidine, the results of this study are in accordance with the study reported by Malmasi and Ghaffari, (2015) but show differences from that reported by Wolfran et al., (2022). The animals used in the study were neutered animals, and this is thought to be possibly related to the higher intraocular pressure due to hormonal effects in non-neutered animals (Ofri et al., 2002).

It is reported that atipemazol use reverses the effects of α-2 adrenoreceptor agonists and reduces IOP to baseline levels (Wolfran et al., 2022). In the present study was in accordance with the results of the study reported by Wolfran et al., (2022).

In conclusion, this study showed that medetomidine hydrochloride and dexmedetomidine administered via intramuscular route decreased intraocular pressure but the levels were within the reference range.

Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept: B.K., Design: B.K., Data Collection or Processing: M.B. and Z.P, Analysis or Interpretation: B.K. and Z.P, Literature Search: B.K. and M.B., Writing: B.K.

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RESEARCH ARTICLE

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The Effect of Different Diseases (*Hepatozoon canis*, Distemper and *Babesia canis canis*) on Serum Haptoglobin, Ceruloplasmin and Albumin Levels in Dogs

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Abstract

In this study; it was aimed to determine serum Hp, Cp, and Alb levels in dogs infected with *Hepatazoon canis*, Distemper and *Babesia canis canis*. The material of the study; 45 dogs infected with *H. canis* (n=15), *B. c. canis* (n=15) by PCR analysis, and canine distemper (n=15) with the rapid diagnosis kit, which were brought to Iğdır University Tuzluca Animal Hospital formed. In addition, 15 healthy dogs were used for control purposes in the study. The serum Hp, Cp, and Alb levels of dogs with *H. canis* were determined as 1.8712±0.003 mg/mL, 9.1746±1.504 mg/dL, and 3.1067±0.073 g/dL, respectively. The serum Hp, Cp, and Alb levels of dogs with distemper were 1.8787±0.005 mg/mL, 7.3016±1.439 mg/dL, and 2.9667±0.080 g/dL, respectively. The serum Hp, Cp, and Alb levels of dogs with *B. c. canis* were measured as 1.8780±0.002 mg/mL, 7.8456±2.092 mg/dL, and 3.2467±0.129 g/dL, respectively. Whereas, serum Hp, Cp, and Alb concentrations of healthy dogs were determined as 1.8662±0.003 mg/mL, 2.9745±0.343 mg/dL, and 2.9600±0.108 g/dL, respectively. While Cp concentration of sick animals were higher than healthy animals ($P<0.05$), there was no statistically significant difference in Hp and Alb concentrations ($P>0.05$). As a result, it was determined that serum Cp concentration increased in dogs with *H. canis*, distemper and *B. c. canis* compared to healthy dogs, while Hp and Alb concentrations did not change.

Keywords: Albumin, *Babesia canis canis,* ceruloplasmin, distemper, dog, haptoglobin, *Hepatazoon canis.*

INTRODUCTION

The stimulation of the neuro-immuno-humoral system as a result of tissue damage and the restoration of the damaged tissue is called acute phase response (APR) (Milanović et al., 2019; Kırmızıgül et al., 2020). In short, APR can also be considered as an early warning system that informs the state of the body (Sevgisunar and Şahinduran, 2014). Proteins synthesized as a result of APR are called acute phase proteins (APP) and are generally produced in hepatocytes and some extrahepatic tissues and organs (adipose tissue, testicular tissue, uterus, ovary, mammary glands, lung, digestive system) (Gökce and Bozukluhan, 2009; Tuna and Ulutaş, 2015). APP are proteins whose serum concentrations increase (positive APP) or decrease (negative APP) by at least 25% in response to injury (Hacımustafaoğlu, 2017). Positive APP's Serum Amyloid A (SAA), Haptoglobin (Hp), C-Reactive Protein (CRP), α-1 Acid Glycoprotein (AGP), Ceruloplasmin (Cp), and Fibrinogen (Fb); Negative APP's are reported as Albumin (Alb), transthyretin (TTR/prealbumin), cortisol binding globulin, retinol-binding protein (RBP) and transferrin (Tf) (Tothova et al., 2014; Iliev and Georgieva, 2018; Erkılıç, 2019).

Haptoglobin is synthesized by the liver and its function is to prevent iron loss by forming stable complexes with free hemoglobin in the blood (Sevgisunar and Şahinduran, 2014; Tuna and Ulutaş, 2015). As a result, Hp limits the availability of iron required for bacterial growth and has a bacteriostatic effect. Also Hp; shows anti-inflammatory properties by binding hemoglobin and integrins (main receptors on the cell wall of leukocytes) (Sevgisunar and Şahinduran, 2014). Serum Hp concentration**s** increase in dogs in cases of inflammation, trauma, and infection (Mcgrotty et al., 2003; Kırmızıgül et al., 2020). Cp is synthesized in the liver and extrahepatic tissues; it's a protein responsible for the transport of copper in the plasma and also protects the tissues from damage caused by free radicals containing iron. In addition, CP shows antioxidant and cell protective activity (Sevgisunar and Şahinduran, 2014; Erkılıç, 2019). In acute phase reactions; Alb, which is one of the negative acute phase proteins, is synthesized by the liver and its most important task is to keep the plasma oncotic pressure in balance (Tuna and Ulutaş, 2015; Kırmızıgül et al., 2020).

Hepatozoon canis is an apicomplexan parasite of the family Hepatozoidae and is transmitted by the brown dog ticks, *Rhipicephalus sanguineus* (*R. sangeineus*) (Baneth, 2011; Aktaş et al., 2015). *H. canis* causes long-term parasitemia clinically (Baneth, 2011). In cases of high parasitemia, symptoms such as fever, anorexia, weight loss, anemia, ocular discharge, and paralysis of the hind legs are occured (Baneth et al., 2001).

Canine distemper; it's a highly contagious, multisystemic, and often fatal viral disease that affects domestic and wild dogs as well as other terrestrial and aquatic carnivores (Gallina et al., 2011). Canine distemper

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virus (CDV) can infect animals in all ages, but animals 0-6 months old are more susceptible (Çalışkan and Burgu, 2007).

Canine babesiosis is a protozoan disease that is transmitted to dogs by ticks, caused by protozoa of the Babesia species, and can cause fatal results (Boozer and Macintire, 2003; Kırmızıgül et al., 2020). Disease agents settle into the erythrocytes and cause the lysis of erythrocytes. Infection occurs in domestic dogs and wild carnivores and is common throughout the world (Gökçe et al., 2013; Sudhakara Reddy et al., 2016; Kırmızıgül et al., 2020). Canine Babesia species are classified into two groups as large and small, according to the morphology of their pyroplasmic forms (Vichova et al., 2016). Large Babesia species have been identified *B. c. canis, B. c. rossi* and *B. c. vogeli* (Uilenberg, 1989; Hauschild, 1995). In addition, an unnamed Babesia species that is closely related to *B. bigemina* has been described in North Carolina in the United States (Boozer and Macintire, 2003). Small Babesia species can infect canines, *B. gibsoni, B. conradae* and *B. microti-like spp.* (Zahler et al., 2000; Baneth et al., 2015). The clinical features of canine babesiosis often include hyperthermia, anemia, hemoglobinuria, lethargy, and anorexia (Bourdoiseau, 2006), but clinical signs in dogs may vary depending on the pathogen (Schetters et al., 1997) and host immunity (Brandão et al., 2003).

In this study; it was aimed to determine serum Hp, Cp and Alb concentrations in dogs with *H. canis*, Distemper and *B. c. canis*. In this way, it will be possible to present the changes in Hp, Cp and Alb concentrations in different diseases seen in dogs.

MATERIALS AND METHODS

Animals

The material of the study consisted of 45 dogs diagnosed with *H. canis* (n=15), *B. c. canis* (n=15), Canine Distemper (n=15) and 15 healthy dogs were brought to Iğdır University Tuzluca Animal Hospital. 8 mL of blood was collected from *V. cephalica* of sick and healthy animals into EDTA and serum tubes. Serum was used for serological and virological analysis, and whole blood sample was used for molecular analysis. For serological analysis, blood samples were centrifuged at 3000 x g for 10 minutes, and serums were separated and stored at -20ºC until Hp, Cp, and Alb concentrations were measured.

Genomic DNA isolation

DNA extractions were performed using the blood DNA extraction kit (Quick-DNATM Miniprep Kit, Zymo Research, USA) according to the manufacturer's instructions. The isolated gDNA was stored at -20ºC until analysis.

Diagnosis of dogs infected with H. canis by PCR

PCR was performed using *H. canis* specific primers HepF (5'-ATA CAT GAG CAA AAT CTC AAC-3') and HepR (5'-CTT ATT ATT CCA TGC TGC AG-3'), which amplify the 666 bp fragment of the 18S rRNA gene (Inokuma et al., 2002; Aktaş et al., 2015; Barati and Razmi, 2018; Akyüz et al., 2020). A 25 µL solution (8,5 µL nuclease-free water, 12,5 µL master mix (Mytaq, Bioline), forward (Hep F) and reverse (Hep R) primers (20 pmol/µL) 1 µL, and 2 µL template DNA) was used for PCR using a thermal cycler (Biometra, Analytik Jena, USA). Positive and negative control DNA samples were used for each reaction. PCR conditions; an initial 5 min denaturation at 95ºC; 34 repeated cycles of denaturation (95ºC for 30 s), annealing (57ºC for 30 s), and extension (72ºC for 90 s); followed by a 5 min extension at 72ºC (Barati and Razmi, 2018; Akyüz et al., 2020).

Diagnosis of dogs infected with B. c. canis by PCR

Confirmation of the species in the cases examined was performed by PCR using *B. c. canis* specific primers. The Bab1(5'-GTG AAC CTT ATC ACT TAA AGG-3') and Bab3 (5'-CTA CAC AGA GCA CAC AGC C-3') primers belong to 18S rRNA gene region (Duarte et al., 2008) which amplify the 746 bp region were used in PCR. A 25 µL solution (8,5 µL nuclease-free water, 12,5 µL master mix (Mytaq, Bioline), forward (Bab1) and reverse (Bab3) primers (20 pmol/µL) 1 µL, and 2 µL template DNA) was used for PCR using a thermal cycler (Biometra, Analytik Jena, USA). Positive and negative control DNA samples were used for each reaction. PCR conditions; an initial 2 min denaturation at 94ºC; 35 repeated cycles of denaturation (94ºC for 30 s), annealing (56°C for 30 s), and extension (72ºC for 45 s); followed by a 10 min extension at 72ºC (Duarte et al., 2008; Gokce et al., 2013).

Running out and Visualizing

PCR products were run out on a 1.5% agarose gel using 0.5X TAE and visualized by ethidium bromide (0.5 µg/mL) stain under ultraviolet light.

Diagnosing dogs with canine Distemper

A rapid diagnosis kit (ASAN Easy Test, Canine Distemper Virus Antigen Test, Cat. No: 022321, Korea, relative sensitivity: 97,96%) was used to identify dogs infected with distemper. The test kit was used in according to the manufacturer's recommendations. Eye conjunctival secretion and nasal discharge samples were taken from dogs suspected of distemper with a swab. The samples were mixed with the dilution solution until dissolved. 3-4 drops (approximately 100 µL) of the solution thoroughly mixed with the samples were dropped into the sample chamber of the disposable cassette. The rapid test kit result was evaluated within 5-10 minutes. In the area following the reservoir; those with control and test lines were evaluated as distemper positive, and those with only control lines were evaluated as distemper negative.

Serological analysis

Hp and Cp concentrations in serum samples were determined using the ELISA device (Thermo Scientific Multiscan GO, TYPE: 1510) and the commercial Elisa kit (BT LAB, China). Alb level was determined with a fully automatic analyzer device (Mindray BS-120).

Statistical analysis

SPSS 20 package program was used for statistical analysis of the obtained data. Shapiro-Wilk test ($n<50$) was used to test whether the data showed normal distribution and it was determined that it showed normal distribution ($P>0.05$). One-way Analysis of Variance (ANOVA), a parametric test, was used to determine whether there was a difference between the groups, and the Turkey test was used to determine the difference between the groups. All results were given as Mean±SE.

RESULTS

Haptoglobin, Cp and Alb concentrations in the serum of dogs infected *H. canis*, Distemper, *B. c. canis*, and healthy dogs are given in Table 1.

Table 1 Serum Hp, Cp and Alb Concentrations of *H. canis*, Distemper and *B. c. canis* in Dogs (n=15)

Parameters	Group	Mean±SE (Min-Max)	F/P
Hp (mg/mL)	Distemper	1.8787±0.005[a] (1.85-1.93)	F=2.381 P=0.079 P>0.05
	H. canis	1.8712±0.003[a] (1.84-1.89)	
	B. c. canis	1.8780±0.002[a] (1.86-1.90)	
	Healthy	1.8662±0.003[a] (1.85-1.90)	
Cp (mg/dL)	Distemper	7.3016±1.439[b] (1.47-16.06)	F=3.264 P=0.028 P<0.05
	H. canis	9.1746±1.504[b] (2.58-25.43)	
	B. c. canis	7.8456±2.092[b] (1.75-25.43)	
	Healthy	2.9745±0.343[a] (0.05-4.93)	
Alb (g/dL)	Distemper	2.9667±0.080[a] (2.50-3.50)	F=1.832 P=0.152 P>0.05
	H. canis	3.1067±0.073[a] (2.60-3.50)	
	B. c. canis	3.2467±0.129[a] (2.40-4.50)	
	Healthy	2.9600±0.108[a] (2.10-3.70)	

[a,b] The difference between the means shown with different letters in the same column is significant (P<0.05)

The Hp (mg/mL) concentration of canine distemper, *H. canis, B. c. canis* and healthy dogs were determined as 1.8787±0.005, 1.8712±0.003, 1.8780±0.002 and 1.8662±0.003, respectively (Table 1). However, there was no significant difference in Hp concentration between healthy dogs and sick dogs (P>0.05). Likewise, when the Alb (g/dL) concentration of sick and healthy dogs were examined, the averages were 2.9667±0.080, 3.1067±0.073, 3.2467±0.129 and 2.9600±0.108, respectively (Table 1). Nevertheless, there was no significant difference between healthy dogs and sick dogs in terms of Alb concentration (P>0.05).

In the analysis, the Cp (mg/dL) concentration of the sick dogs were 7.3016±1.439, 9.1746±1.504, 7.8456±2.092, respectively and no statistically significant difference was found between the serum Cp concentration of the dogs with canine distemper, *H. canis* and *B. c. canis*. On the other hand, serum Cp concentration of 2.9745±0.343 in healthy dogs was lower than that of sick dogs, and the difference was statistically significant (P<0.05).

DISCUSSION AND CONCLUSION

Acute phase proteins are used in the diagnosis, differential diagnosis, and prognosis of diseases as well as in determining the efficacy of treatment. The concentrations of APP's, which are not specific to the disease, but whose concentrations increase rapidly in cases of tissue destruction and inflammation, decrease with effective treatment. The concentration of the increase in plasma concentrations of APP's correlates with the size and activity of the inflammation. Therefore, the determination of the circulating concentrations of these proteins provides information about the ongoing inflammatory reaction. Profile, synthesis, secretion, and excretion of APP's differ between animal species (Murata et al., 2004; Cecilliani et al., 2012).

Hepatozoon canis; it's transmitted by *R. sanguineus* ticks and causes clinically long-term parasitemia. The disease is endemic in temperate climate zone, tropical and subtropical regions where vector ticks are active (Baneth, 2011). In studies conducted in different parts of the world, the prevalence of *H. canis* has been reported to vary between 7.5-52%. (Rojas et al., 2014; Maia et al.,2015; Piratae et al., 2015; Hamel et al., 2016). In a study, it was reported that the Hp concentration in dogs with *H. canis* was similar to the control group. In the same study, it was reported that the Cp concentration increased statistically significantly (Ulutaş et al., 2007). In another study, it was reported that the concentration of Alb decreased in dogs with *H. canis* (Paşa et al., 2009). In addition, increased Hp concentration have been reported in cats infected with *H. felis* (Vilhena et al., 2017). Moreover; according to the increase levels, positive AFPs are evaluated in 3 groups as those that increase approximately 50% (Cp and complement factor 3), those that increase 2-3 (Hp, fibrinogen, α-globulins with antiprotease activity and lipopolysaccharide binding protein) times and those that increase 5-1000 (CRP and SAA) times (Ulutaş et al., 2008). In the study, it was determined that there was no statistically significant change in Hp and Alb concentrations in dogs with *H. canis*, while the Cp concentration increased approximately 4 times. According to our results in study, although Hp and Cp levels are parallel to the literature data, there is a difference in Alb level. This situation; it can be said that the disease is caused by the severity, care and feeding differences. Because; the entire life cycle of *H. canis* is completed in a total of 81 days in ticks and dogs (Baneth et al., 2007). Moreover; it's possible to encounter the agent in the lung, heart, skeletal muscles, liver, spleen and lymph nodes in the schizogonic period, and low parasitemia is the most common form of infection in which less than 5% of neutrophils are infected (Baneth, 2011).

Canine distemper; it is a highly contagious, multisystemic and often fatal viral disease (Gallina et al., 2011). It has been reported that the prevalence of CDV, which is endemic globally, is up to 71% (Fischer et al., 2016; DiGandi et al., 2019). In a study, it was reported that there was a decrease in the concentration of Alb in dogs infected with CDV, but this situation was not statistically significant (Değirmençay et al., 2021). Kogika et al., (2003); in his study on CDV, he reported slight increases in Hp and Cp levels. In another study, it was reported that Hp and Cp concentrations increased and Alb concentration decreased in CDV-infected dogs (Kocatürk et al., 2011). Moreover, it has been reported that the increase in Cp level in dogs with canine distemper is an important prognostic indicator of the disease (Erogowda et al., 2020). In our study, it was determined that the Cp concentration increased (approximately 2,5 times) significantly parallel with the literature. This suggests that high Cp level protects against hepatic production and tissue damage (Schmidt et al., 2015).However, although there were very small numerical increases in Hp and Alb concentrations, no statistically significant change was observed. The numerical increase in Hp and Alb levels in sick dogs compared to healthy dogs suggests that this may be related to racial differences or nutrition. Moreover; clinical symptoms of CDV infections with an incubation period of 1-3 weeks; it depends on the host's immune system, the state of secondary bacterial infection, and particularly the strain of the virus (De Almeida, 2009; Ludlow et al., 2012).

Babesiosis, the prevalence of which varies between 2.2-67.8% in the world; It is a protozoan disease transmitted to dogs by ticks and can lead to fatal consequences (Boozer and Mcintyre, 2003; Watanabe et al., 2004; Rodriguez-Vivas et al., 2005; Davoust et al., 2006; Tsachev et al., 2006; Kırmızıgül et al., 2020). In a study, it was reported that Alb and Hp concentrations decreased in dogs with *B. c. canis* compared to healthy dogs, while Cp concentration increased (Erkılıç, 2019; Kırmızıgül et al., 2020). In our study, it was determined that there was no statistical change in Alb and Hp concentrations in dogs with *B. c. canis,* while the Cp concentration increased approximately 3 times. It was thought that the reason for the higher Cp concentration in sick dogs compared to healthy dogs was due to the inflammatory changes that occurred. In our study; Hp and Alb concentrations in sick dogs showed slight increases numerically compared to healthy dogs. This situation in Hp and Alb concentrations can be explained as being in the early stages of the disease. Because; it has been reported that Hp and Alb levels decrease as a result of hemolysis in advanced stages of babesiosis (Kırmızıgül et al., 2020).

As a result, with this study; Species-specific Hp, Cp and Alb levels, whose importance in veterinary medicine are increasing day by day, have been determined for diagnosis and differential diagnosis in different diseases (*H. canis*, distemper and *B. c. canis*) in dogs. It was determined that while Cp concentration increased in dogs with *H. canis,* distemper and *B. c. canis* compared to healthy ones, Hp and Alb concentrations did not change. It can be said that the Hp and Alb concentrations in these diseases are similar to those of healthy dogs, which is related to the severity of the disease. Higher Cp concentration in dogs with *H. canis*, distemper, and *B. c. canis* compared to healthy dogs; It is thought to be due to oxidative damage and inflammatory changes. However, it is thought that detailed studies are needed to determine the clinical importance of the prognosis and treatment efficacy of these diseases.

Conflict of Interest
The authors declare that they have no competing interests.

Authorship contributions
Concept: C.A., Design: C.A., Data Collection or Processing: C.A., Ş.K., N.A., Analysis or Interpretation: C.A., Literature Search: C.A., Writing: C.A., Ş.K., N.A.

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Ethical Approval
This study was approved by the Kafkas University Animal Experiments Local Ethics Committee (Approval no: 2022-090).

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RESEARCH ARTICLE

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Retrospective Evaluation of Spinal Trauma Treatments in 58 Cats and 12 Dogs

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Abstract

Traumatic vertebral fractures and/or dislocations in cats and dogs can cause severe spinal cord injury, resulting in severe conditions such as pain, urinary incontinence, paresis or paraplegia. This study involved 58 cats and 12 dogs with external spinal trauma, and it was aimed to present the etiology, treatment and results, retrospectively. After the location of the neurological damage was determined, the patients were treated either conservatively or surgically. One of the surgical methods such as polyaxial screw, locking plate application and external fixation application was decided. In the treated animals, complete recovery was seen in 10 cats and functional recovery in 14 cats and 1 dog, but 12 cats and 2 dogs were in poor condition. Loss of deep pain sensation after spinal trauma is important for prognosis. Although there are many different treatment options, the decision should be made according to the patient's condition and the surgeon's preference. In addition, patients with spinal trauma also require serious trauma management and it is very important that the animal's vital values are stable.

Keywords: Spinal trauma, vertebral surgery, polyaxial screw, locking plate, cat, dog.

INTRODUCTION

In small animal practice, neurological spinal cord diseases such as intervertebral disc disease are not always caused by trauma. However, vertebral fractures and dislocations can cause serious spinal cord injury. This is about 10% of all neurological problems in dogs and cats (Orgonikova et al., 2021). In cats and dogs, traffic accidents, falling from a height and gunshot wounds are among the main causes of spinal trauma (Grasmueck and Steffen, 2004; Bruce et al., 2008; Bali et al., 2009; Ahn et al., 2015; Özak et al., 2018; Diamante et al., 2020; Orgonikova et al., 2021; Caterino et al., 2022). The most common spinal injuries are seen in the lumbar vertebrae, followed by the sacrococcygeal, thoracic and cervical vertebrae. Also, a high incidence of fractures or luxation at the thoracolumbar and lumbosacral junctions has been reported (Bruce et al., 2008; Diamante et al., 2020). Spinal cord injuries can cause serious conditions ranging from localized pain to paresis, paraplegia or tetraplegia (Kirby 2010; Negrin and Cherubini 2016; Inglez et al., 2017).

It is recommended to approach a patient with spinal trauma in three steps (Sulla et al., 2019; Orgonikova et al., 2021). These;

Step 1: Airways-breathing-circulation (ABC) assessment, stabilization and immobilization
Step 2: Neurological examination
Step 3: Analgesia, sedation, or anxiolysis.

Treatment depends on the patient's signal, the nature of the injury, the neurological status, and the individual surgeon's experience (Bruce et al., 2008). Treatment is generally considered conservative and surgical. Conservative treatment typically consists of splints and bandages, cage confinement, exercise restriction, and administration of steroids. In surgical treatment, decompression of the spinal cord and rigid stabilization of the spinal canal are aimed. For this purpose, various fastening systems such as pins and polymethylmethacrylate (PMMA), screws and plates are used (Bruce et al., 2008; Kirby, 2010; Özak et al., 2018; Sulla et al., 2019). Because of the poor prognosis and persistent suffering of the severely affected animal, many owners consider euthanasia (Sulla et al., 2019).

In this study, it was aimed to present the etiology, treatment and results retrospectively in cats and dogs with spinal trauma.

MATERIALS AND METHODS

This study was carried out in Dicle University Veterinary Faculty surgery clinics between 2019 and 2022. 58 cats and 12 dogs with spinal trauma were included in the study and analyzed, retrospectively. The case inclusion was accepted based on clinical and neurological records, radiographic data and treatment information. The neurological diagnosis was based on clinical, neurological, radiographic examination and intraoperative findings. The data obtained from the records were focused on the etiology, neurological and radiographic findings and treatment (non-surgical/surgical). While surgical treatment included dorsal laminectomy and hemilaminectomy for decompression, it consisted of the use of a polyaxial screw, locking plate and external fixator for vertebral stabilization. After the treatment, it was

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followed up with phone calls and repeated neurological examinations on a weekly basis. For at least four weeks, a visible improvement was observed. There were cases with a follow-up period of four weeks to two years. At the end of the study, cases were clinically classified as complete, functional, or poor (as in the previously reported studies, Grasmueck and Steffen, 2004).

In addition to the age, sex and breed information of cats and dogs, the localization of a detected condition such as vertebral fracture, luxation, or compression was recorded. Common causes included trauma information such as traffic accidents, falls from height, other animal attacks, and gunshot wounds. It was also clinically graded into five groups according to the severity of their neurological dysfunction as Grasmueck and Steffen, (2004) (Table 1).

Table 1. Grades according to the severity of neurological symptoms as Grasmueck and Steffen, (2004).

Grade I	Back pain, no neurological deficits	5 cats, 1 dog
Grade II	Ambulatory paraparesis, normal micturition	7 cats
Grade III	Ambulatory paraparesis, urinary retention	14 cats, 3 dogs
Grade IV	Non-ambulatory paraparesis/paraplegia, urinary retention, intact deep pain perception	15 cats,
Grade V	Paraplegia, urinary retention, loss of deep pain perception	17 cats, 8 dogs

In order to evaluate the perception of deep pain, the extremities and tail were clamped with forceps, while the head region of the animal, especially the eyes, was followed well. Deep pain perception of cats with suspicious responses to the stimulus was considered negative.

Radiographs were taken in latero-lateral and ventro-dorsal positions in all cases. In suspicious cases, myelography was performed by entering the subarachnoid space with cisternal or lumbar puncture under general anesthesia. For myelography, iohexol 0.3 ml/kg body weight (Omnipaque^{TM} 350; Opakim-İstanbul) was injected via a 20-22 G spinal needle.

In terms of the treatment group, the patients are categorized as conservative and surgical. Conservative treatment consisted of cage rest and exercise restriction. Surgical techniques were polyaxial screwing, locking plates and external fixator, which are among the stabilization techniques (Figure 1-3). Dorsal laminectomy or hemilaminectomy was also performed for decompression (Table 2). Treatment groups were not randomized and treatment decisions were made based on clinical judgment, clinician preference, as well as owner financial status.

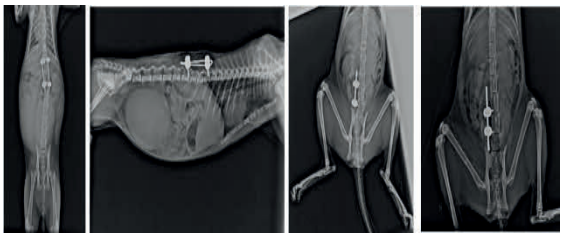

Figure 1. Examples of cases using polyaxial screwing; The first two images on the left belong to the same case, the rod used to fix the polyaxial screws was used reciprocally, while in the cases in the other two images, one-sided rods were used.

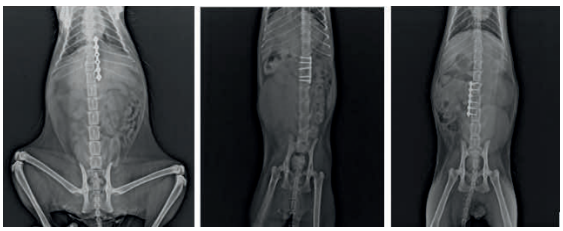

Figure 2. A few examples of cases where SOP plates were used for vertebral stabilization; It is more beneficial to provide stabilization with one anterior and one posterior vertebra, especially in end-plate fractures.

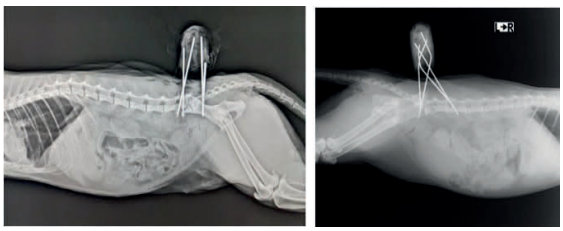

Figure 3. The use of external fixators for vertebral stabilization is not very common for this subject, but can be particularly useful for caudal vertebrae.

Table 2. Localization of trauma and planned treatment in cases.

Animals	**Gender**	**Localization**					**Treatment group**					
							Non-surgical	**Surgical**				
								For decompression		For stabilization		
		C1-C5	C6-T2	T3-L3	L4-L7	S1-S3		DL	HL	PS	LP	EF
Cats	37 F	-	2	16	18	1	4	13	5	12	8	4
(n=58)	21 M	-	-	11	10	-	1	9	2	6	4	2
Dogs	4 F	1	1	2	-	-	4	-	-	-	-	-
(n=12)	8 M	-	-	2	6	1	1	3	-	1	2	-

DL: Dorsal laminectomy, HL: Hemilaminectomy, PS: Polyaxial screwing, LP: Locking plates, EF: External fixator. - During the operation, decompression was achieved first, followed by vertebral stabilization.

Among the patients who were planned for surgery, autologous plasma-rich platelets (PRP) were applied to the spinal cord during the operation in those who underwent dorsal laminectomy or hemilaminectomy. After deciding whether all blood samples were suitable for platelet, white blood cell (WBC) and hematocrit (Hct) analysis according to the hematology laboratory results, PRP was prepared by double centrifugation technique as previously reported (Arican et al., 2018).

Most of the cases (50 cats-86%, 10 dogs 83%) could be followed up after treatment to evaluate clinical outcomes. Persistent difficulty in urination and/or lack of improvement in walking after treatment was defined as a poor outcome. Bladder control and the ability to walk unaided and painless were considered functional improvement. Complete recovery was normal urination, normal gait and absence of proprioceptive abnormalities.

RESULTS

All patients (58 cats and 12 dogs) had varying degrees of neurological dysfunction. This neurological dysfunction distribution was determined as 5 cats and 1 dog in grade I, 7 cats in grade II, 14 cats 3 dogs in grade III, and 15 cats in grade IV, and 17 cats and 8 dogs in grade V (Table 1). Urinary retention was observed in 46 cats (79%) and 11 dogs (91%).

In terms of the affected area, respectively, L4-L7 (28 cats-48%, 6 dogs-54%), T3-L3 (27 cats 46%, 4 dogs-36%), C6-T2 (2 cats-3%, 1 dog- 1%), S1-S3 (1 cat-1%, 1 dog-1%) (Table 2).

In cats with grade II and III neurological dysfunction (n=21), the outcome was poor in five, functional recovery in nine, and complete recovery in seven. In dogs with grade III neurologic dysfunction (n=3), two had poor results and one had functional improvement. In cats with grade IV neurological dysfunction (n=15), recovery was weak in seven, functional in five, and complete in three. In all cats (n=17) and dogs (n=8) classified as having grade V neurological dysfunction, no treatment was applied by consensus of the owner.

Of the five cats treated non-surgically, three recovered completely, one functionally recovered and one had poor outcomes. While 1 out of 5 dogs fully recovered, 1 recovered functionally, and there was no news from the others (3 dogs). In this group, animals were treated only with cage rest and rehabilitation treatment without the use of steroids.

According to the data in the study, in terms of trauma; there were 49 falls from height, 7 traffic accidents and 2 unknown cases in cats. The cause of spinal trauma in dogs was a traffic accident in six, while the cause of the other six was unknown.

DISCUSSION AND CONCLUSION

Traumatic spinal cord injuries in cats and dogs are still an important issue. Although medical and/or surgical treatment protocols have been defined in the literature, clinical data are limited. In addition, it is reported that paraplegic cases with spontaneous herniation of degenerated discs or spinal cord injury in traumatic situations have a good prognosis provided that timely decompression is performed (Grasmueck and Steffen, 2004; Bali et al., 2009). Therefore, in this clinical study conducted on 58 cats and 12 dogs, it was aimed to retrospectively report the clinical results of spinal trauma patient management and treatments to veterinary practice.

Mostly, traffic accidents, falls from height, gunshot wounds and other animal attacks are among the causes of spinal trauma (Grasmueck and Steffen, 2004; Bruce et al., 2008; Bali et al., 2009; Ahn et al., 2015; Gönenci et al., 2017; Özak et al., 2018; Diamante et al., 2020; Orgonikova et al., 2021; Caterino et al., 2022). In our study, it was observed that falling from a height (84%) and traffic accidents (12%) were effective in cats in general, and traffic accidents (50%) were effective in dogs. Vertebral fracture or luxation depends on the severity of the trauma forces. However, in this study, neither cats nor dogs found a remarkable association with the cause of the trauma in terms of severity of the injury, location of the lesion, presence of multiple lesions, or incidence of complications.

Vertebral fracture or luxation usually occurs in the vertebrae (corpus or end plate) or in the joint between the vertebrae, close to the skull, thorax, and pelvis. The terminal thoracic region is the most commonly affected area in the dog. Most of the lesions in the thoracolumbar region were reported with similar results in dogs (58%) and cats (49%). It has been reported that the closest region to this is the lumbar region (L1-L7) and then the sacro-caudal region (S1-Cc3) (Bali et al., 2009; Grasmueck and Steffen 2004). In our study, the rate of injury at L4-L7 (28 cats-48%) and T3-L3 (27 cats 46%) levels were found to be very close to each other. This was followed by C6-T2 (2 cats-36) and S1-S3 (1 cat-1%). Although the number of cases was limited in dogs, similar results were found as in cats (L4-L7; 6 dogs-54%, T3-L3; 4 dogs-36%), C6-T2; 1 dog- 1%, S1-S3; 1 dog-1%).

Animals with spinal trauma and vertebral fracture or luxation may have spinal discomfort, paresis or paralysis, depending on the location and severity of the injury. These are similar to those in patients with other spinal cord lesions such as intervertebral disc disease and fibrocartilaginous embolism (Orgonikova et al., 2021). A rapid scan is initially sufficient to evaluate an animal with a spinal fracture or luxation. A complete neurologic examination may not initially be necessary to avoid possible iatrogenic damage to the patient's manipulation during the postural reaction test. The neurological examination aims to localize the lesion(s) affecting the nervous system and determine its extent and severity. Initial grading is the most important clinical feature that affects the prognosis or treatment outcome of a spinal cord injury (Grasmueck and Steffen 2004; Orgonikova et al., 2021).

In patients with spinal trauma, the presence of deep pain sensation following spinal trauma is one of the most important findings for prognosis. Because lack of perception of deep pain associated with luxation and fractured vertebra means a poor prognosis. In addition, urinary and/or fecal incontinence, perineal reflex, deep pain sensation in genital, anal and tail should be evaluated in spinal traumas (Negrin and Cherubini, 2016). A second spinal injury has been reported in approximately 20% of patients with thoracolumbar fractures (Grasmueck and Steffen, 2004); therefore, CT or MRI imaging of all vertebrae should be performed in spinal traumas (Negrin and Cherubini, 2016). In our study, a detailed neurological examination was performed in all animals, and urinary or fecal incontinence was examined as well as deep pain sensation. Urinary incontinence was seen in 77% of cats (n=45) and 91% of dogs (n=11). Deep pain sensation loss was seen in 17 cats (29%) and 8 dogs (66%). It is obvious

that a paraplegic animal does not have a comfortable life. In addition, it is very difficult to care for an animal with urinary incontinence at home, and patient owners often have difficulties. On the other hand, CT could not be used in our study because we did not have such an opportunity. However, myelography was performed alongside direct radiography. In addition, we did not do epidurography, but epidurography could have been done for a similar purpose.

Systemic stabilization is important in the management of a patient with spinal trauma, and evaluation of the airway, respiration and circulation is essential (Eminaga et al., 2011). HCT, total protein level, urea and creatinine concentration, and electrolyte balance should be checked as soon as possible. Fluid therapy is important to maintain spinal cord perfusion, depending on the severity of hypotension, isotonic crystalloids, hypertonic saline, colloids, or blood products can be used (Eminaga et al., 2011; Negrin and Cherubini, 2016). As in all trauma patients, the "airways-breathing-circulation (ABC)" evaluation in spinal trauma patients should be considered by the trauma protocol, and this was also done in our study. If the patient was stable, a detailed neurological examination was subsequently performed.

Another topic of discussion is the use of steroids in acute spinal trauma. Because it can cause secondary side effects, including infection and gastrointestinal symptoms (Grasmueck and Steffen, 2004; Negrin and Cherubini, 2016). Steroid administration was not used in our study.

Platelet-rich plasma (PRP) is a concentrated source of autologous platelets in plasma. Since PRP contains autologous growth factors that accelerate tissue healing, it is known to be used in different areas such as wound healing, peripheral nerve injuries, and after plastic surgery. In addition, there are animal studies on its applications in the spinal cord (Chen et al., 2018). In our study, PRP was prepared and applied locally on the spinal cord in the form of drops in all of the cases who underwent laminectomy. This study was not conducted to directly evaluate the efficacy of PRP on spinal cord damage/healing, but we think that PRP application will also be beneficial if blood values are appropriate and spinal cord decompression will be performed.

Although many different techniques are used for stabilization in vertebral fractures or luxations, some of the applications that find common use among these are the use of polyaxial screws, stabilization with polymethylmethacrylate (PMMA) and plate applications (Grasmueck and Steffen, 2004; Negrin and Cherubini, 2016; Özak et al., 2018; Diamante et al., 2020; Orgonikova et al., 2021). In our study, fixation with polyaxial screwing (18 cats, 1 dog), locking plate (nearly all of them consisted of String of Pearls plate, SOP plate) (12 cats, 2 dogs) and external fixator (6 cats) techniques were used. Which of these methods will be used is completely determined by the patient's condition and the surgeon's decision. It is difficult to say which of the techniques is more effective according to the fracture structure. They are methods that really give results when applied well. We did not encounter any complications arising from any preferred method itself. In addition, dorsal laminectomy or hemilaminectomy techniques have been described for decompression of the vertebral column, and decompression is required (Özak et al., 2018; Orgonikova et al., 2021). In our study, an electro-power device with a diamond milling tip was used to open the lamina during laminectomies. Afterward, he worked with kerrison rongeur measuring 0.8 mm for cats and 1.2 mm for dogs. We recommend using a kerrison rongeur instead of forceps such as rongeur to work in favor of the spinal cord during laminectomy.

In paraplegic cats, the intact sense of deep pain is very important in terms of neurological damage and treatment prognosis. The spinal trauma patient is also a trauma patient. It requires trauma management and it is very important that the vital organs of the animal are stable. Subsequently requires a good neurological examination and needs to make a good treatment decision. There are different stabilization methods preferred for treatment, and this is entirely dependent on the surgeon's choice, set inventory, and patient's condition. Even if there is no complete clinical recovery, animals with functional recovery can continue their lives more comfortably at home.

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Conflict of Interest
The authors declare that they have no competing interests.

Authorship contributions
Concept: S.Y., Design: S.Y., S.A., Data Collection or Processing: N.S., M.K., Analysis or Interpretation: E.Ç., Literature Search: B.E.K., Writing: S.Y.

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Ethical Approval
All methods and procedures used in this study comply with the guidelines of the Turkey and EU directive (Directive 2010/63/EU) on the protection of animals used for scientific purposes. This study did not require approval from the authorities or the ethics committee of the institution. However, patient owners were informed and consent was obtained.

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REVIEW ARTICLE

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Brachycephalic Airway Syndrome in Dogs

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Abstract

Brachycephalic dogs are characterized by a shortened muzzle of varying sizes and a round and large head. These breeds include the Boston Terrier, English Bulldog, French Bulldog, Pug, Pekingese, Shih Tzu, and Cavalier King Charles Spaniel. Brachycephalic airway syndrome is a common finding in brachycephalic breeds. A combination of primary and secondary changes can progress to life-threatening laryngeal collapse. In this review, information will be given about narrow nostrils, elongated soft palate, and everted laryngeal saccules, which are brachycephalic primary respiratory tract pathologies.

Keywords: Brachycephaly, airway syndrome, diagnosis, anestesia, treatment.

INTRODUCTION

In the world is known that there are close to 400 types of dogs. Significant differences in morphology and especially in skull shapes were observed among these species (Schoenebeck and Ostrander, 2013). The skull sizes of these breeds are observed to vary between 7 and 28 cm (McGreevy et al., 2004). It is known that three terms as dolichocephalic, mesocephalic and brachycephalic are generally used to describe skull shapes (Roberts et al., 2010). This classification is made according to the cephalic index calculated in dogs (Helton, 2009).

Brachycephalic breeds are generally distinguished from mesocephalic and dolichocephalic races by the presence of short skulls and open orbitals. Brachycephalic dog breeds include the Boston Terrier, English Bulldog, French Bulldog, Pug, Pekingese, Shih Tzu, and Cavalier King Charles Spaniel (Dupré et al., 2012). A definitive list of the brachycephalic breeds is not available because there are differences in definitions of 'brachycephaly'. The variation within some breeds indicates that the term is most appropriate for individual consideration of dogs rather than breeds as a whole (Ekenstedt et al., 2020).

The aim of this review is to give information about the anatomy, pathophysiology, diagnosis and treatment of the brachycephalic primary respiratory tract pathologies in dogs, which are narrow nostrils, elongated soft palate and everted laryngeal saccules.

Anatomy

Brachycephalic dogs are characterized by a shortened muzzle of varying sizes and a round and large head. Compared to mesocephalic dogs, brachycephalic dogs; medio-lateral enlargement of the skull appears to be accompanied by rostro-caudal shortening of the nose (Ekenstedt et al., 2020). One of the skull changes in brachycephalic dogs is early ankylosis of the basic cranial epiphyseal cartilage of the skull. This condition leads to chondrodysplasia of the longitudinal axis of the skull. There is currently no consensus on which measurements are standard. However, it is known that the cranio-facial angle of the skull in brachycephalic dogs is 9º-14º (Meola, 2013).

Dorsal and ventral cartilago nasi lateralis join laterally to form the nostril. The nostrils are supported medially and ventrally by the septum nasi and dorsally by the dorsal cartilago nasi lateralis (Fossum, 2007). The alae nasi contains the maxillary levator labii and nasolabial levator muscle fibers and allows this region to expand (Koch et al., 2003).

The soft palate extends from the hard palate to the tip of the epiglottis, effectively separating the oropharynx from the nasopharynx. The musculus palatinus, supplied by the plexus pharyngealis, shortens the soft palate during contraction. The epiglottis is a curved triangular cartilage at the entrance to the larynx. The apex of the epiglottis is towards the oropharynx and is located immediately dorsal to the soft palate. The mucosa forms the plica aryepiglottica by attaching the sides of the epiglottis to the processus cuneiformis of the cartilago arytenoidea (Fossum, 2007).

Pathophysiology

The airflow passing through the nasal cavities constitutes 76.5% of the total airflow resistance in the respiratory tract. In normal dogs, inspiration and expiration are passive (Koch et al., 2003). However, brachycephalic dogs have difficulties due to pathological problems in the respiratory tract. Brachycephalic dogs have an increased resistance to airflow during inspiration and an increased intraluminal pressure gradient due to anatomical differences (Hobsan, 1995). Poiseuille's law states that a 50% reduction in radius results in a 16 fold increase in flow resistance. Therefore, reducing the size of the nostrils, nasal passage and laryngeal openings by half will increase the airway resistance in the brachycephalic breed by 16 fold that of a non-brachycephalic dog. The increased negative pressure created to overcome the resistance causes inflammation of the soft tissue, eversion of the

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laryngeal vesicles and many pathologies such as laryngeal and tracheal collapse (Meola, 2013).

Upper Respiratory Track Pathologies Associed With Brachycephalic Breeds

Brachycephalic airway syndrome is characterized primarily by respiratory and thermoregulatory problems, especially among pathologies involving partial or complete obstruction of the upper respiratory tract (Ekenstedt et al., 2020). Brachycephalic airway syndrome was at first thought to be caused only by narrow nostrils and a long soft palate. Recently, new endoscopic, radiological and computed tomography studies show that most airway obstruction is caused by more than one thing (Table 1) (Grand and Bureau, 2011).

Table 1. Upper airway abnormalities associated with brachycephalic breeds (Fossum, 2007).

Classic Components of Brachycephalic Syndrome
Elongated soft palate
Stenotic nares
Everted laryngeal saccules
Common Concurrent Findings
Hypoplastic trachea
Aryepiglottic collapse
Other Findings
Corniculate collapse
Tracheal collapse
Tonsil eversion
Macroglossia
Pharyngeal collapse
Epiglottic collapse

Stenotic Nares

Stenotic nostrils are congenital deformities that cause medial collapse of the nasal cartilages and partial occlusion of the external nostrils. (Fossum, 2007). The external view of the nostril opening between the nasal septum and the dorsal cartilago nasi lateralis and ventral cartilago nasi lateralis has turned into a vertical slit (Monnet, 2013). Stenotic nostrils have been recognized as the primary anatomical components of the brachycephalic syndrome, and their early correction has been advocated to minimize the increase of obstructive disease in other regions (Tobias, 2017).

Elongated Solf Palate

Elongated soft palate is one of the most common pathologies of brachycephalic syndrome. The elongated soft palate is pulled caudally during inspiration and occludes the dorsal glottis. The laryngeal mucosa is inflamed and edematous, further narrowing the airway. Affected dogs may have difficulty swallowing due to normal occlusion of the airway during swallowing. Dysfunctional swallowing can lead to aspiration pneumonia (Fossum, 2007). Although elongated soft palate has been emphasized as the primary component of brachycephalic syndrome in the literature, recent radiographic, computed tomography (CT) and histological examinations have shown an additional pathological thickening of the soft palate. Another study showed that there is a positive relationship between soft palate thickness and the severity of clinical symptoms (Dupré and Heidenreich, 2016).

Laryngeal Diseases

Laryngeal diseases associated with brachycephalic syndrome include mucosal edema, everted laryngeal vesicles and laryngeal collapse (Koch et al., 2003). Eversion of the laryngeal vesicles has been accepted as the first degree of laryngeal collapse (Leonard, 1960). Eversion of laryngeal vesicles is less commonly diagnosed than elongated soft palate or stenotic nostrils, but has been reported in 58% to 66% of dogs with brachycephalic syndrome (Fasanella et al., 2010). The increase in airflow resistance and the increase in negative pressure to push air through the occluded areas pull the diverticula through their crypts, causing them to swell. These diverticulum obstruct the ventral side of the glottis, further obstructing airflow and thus causing respiratory tract abnormality (Fossum, 2007).

Laryngeal collapse is a form of upper airway obstruction that causes medial collapse of the rostral laryngeal cartilages due to loss of cartilage rigidity. (White, 2012). It has been reported that laryngeal collapse begins in brachycephalic dogs when they are younger than 6 months old (MacPhail, 2019).

DIAGNOSIS

Clinical Appearance

Typical clinical signs in dogs with brachycephalic airway syndrome include; inspiratory stertore, inspiratory stridor, snoring, cough, exercise intolerance, increased respiratory effort, hyperthermia and collapse (Meola, 2013). Rapid breathing increases congestion with stress. Failure to provide normal thermoregulation predisposes dogs to hyperthermia, which can lead to heat stress and death. Any physiological condition can aggravate these conditions and lead to respiratory crises (Ekenstedt et al., 2020).

Physical Examination Findings

During the physical examination, care should be taken not to stress the animal and thus not to increase the respiratory problem. Because even an apparently stable brachycephalic dog can suddenly decompensate (Hendricks, 1992). Examination should first begin with inspection. The paradoxical movement of the thorax and abdomen, the exercise of the accessory respiratory muscles, the inward collapse of the intercostal spaces and the thoracic inlet and the forward extended head and neck position (orthoplenic body position) can be clearly seen (Fossum, 2007). Body temperature should be determined and if the body temperature is high, it should be brought to normal body temperature (Hendricks, 1992).

Gastrointestinal distention with air due to aerophagia is a common symptom accompanying respiratory problem. As the primary syndrome, gastric dilatation and volvulus can also occur in brachycephalics, so it should be differentiated from simple aerophagia (Hendricks, 1992).

Laboratory Findings

It provides assessment of blood gas, hypoxia and respiratory alkalosis. (Fossum, 2007). A pink coloration of the mucosa does not mean it is normal, as cyanosis is usually not detected until the oxygen saturation (SaO_2) is below 80%. Thus, an animal with a SaO_2 between 80% and 90% has pink mucous membranes, although it is at risk for clinically significant reductions. Colour of the mucous membrane; general posture should be evaluated together with the breathing pattern and the use of auxiliary muscles

(Lodato and Hedlund, 2012). However, the safest procedure is to provide oxygen with a face mask or oxygen cage until the partial arterial oxygen pressure (PaO_2) is directly measured (Hendricks, 1992). If arterial blood gas cannot be obtained, a venous sample or pulse oximetry can be used to establish pH and bicarbonate levels and partial pressure of carbon dioxide (Lodato and Hedlund, 2012).

Imaging Diagnostic Methods

Appropriate assessment of the airway; includes many imaging diagnostic methods such as radiography, computer tomography and endoscopy (Dupré et al., 2012). Radiography in brachycephalic respiratory tract pathologies; lateral head and neck X-ray allows to evaluate the degree of upper airway obstruction (Hendricks, 1992). Laterolateral neck radiography allows the evaluation of the thickness of the soft palate located between the nasopharynx and the oropharynx (Dupré and Heidenreich, 2016). Thoracic radiography taken in the laterolateral position, shows us the presence of hypoplastic trachea. The measure of the diameter of the trachea; where the thoracic inlet line cuts the trachea, is the tracheal lumen vertical to the long axis of the trachea. The trachea is considered hypoplastic when the trachea diameter/thoracic entry line ratio is <0.16 on a lateral thoracic radiograph. This measurement is not affected by the respiratory phase. In addition, the size of the trachea diameter can be evaluated by measuring the diameter of the trachea in the direction of the third costa and the diameter of the third costa. If the diameter of the trachea is three times the width of the third costa, it is considered normal (Lodato and Hedlund, 2012).

Computed tomography (CT) in brachycephalic respiratory tract pathologies; It is a powerful and fast imaging method that uses the nose, paranasal sinuses, skull and trachea to create cross-sectional images (Kuehn, 2006). Endoscopy is one of the most used auxiliary diagnostic methods, although no bone can be used to define the location or dimensions of a pathological or anatomical structure (Liu et al., 2018). Endoscopic examination of the airways in brachycephalic airway syndrome includes direct and retrograde rhinoscopy, laryngoscopy and tracheo-bronchoscopy. Rhinoscopy is usually performed to detect vestibular stenosis and turbinate malformation (Dupré et al., 2012). Examination of the pharyngeal and laryngeal structures, usually by endoscopy, is best done under general anesthesia (Ekenstedt et al., 2020). Laryngoscopy is done after the dog is extubated. In this visual diagnosis method, soft palate hyperplasia can be diagnosed first (Fossum, 2007). By pressing the soft palate, everted laryngeal vesicles and vestibular folds may appear, and at the same time movements of the rima glottis can be observed during inspiration and expiration. Tracheal collapse can also be seen in some brachycephalic dogs as a result of continued negative pressure (Koch et al., 2003).

Prognosis

It is difficult to obtain an accurate prognostic perspective for dogs with brachycephalic respiratory syndrome. Most studies evaluating outcome after brachycephalic surgery are retrospective and compare results in different races with various treatment combinations and reconstructive techniques (Dupré and Heidenreich, 2016). It has been found that English Bulldogs respond worse to the operation than all other breeds and are more likely to develop aspiration pneumonia postoperatively. Its relationship with gastrointestinal diseases was investigated, and it was reported that better results were obtained in brachycephalic dogs treated simultaneously with upper respiratory tract diseases (Poncet et al., 2006).

TREATMENT

Medical Treatment

Long-term conservative medical treatment includes a weight management program and intervention in the dog's lifestyle. This is a clinically recognized issue, although one study failed to correlate increased body weight with the severity of respiratory symptoms (Trappler and Moore, 2011a). Activities that increase respiratory effort, including long walks with a collar on during the hottest hours of the day, should be avoided. Walks should be kept short and done during cool hours of the day (Trappler and Moore, 2011a; Meola 2013). Exercise restriction and elimination of triggers can eliminate these clinical symptoms when clinical symptoms are mild. Sedation, corticosteroids, supplemental oxygen and antipyretics to lower body temperature may be necessary for moderate to severe respiratory distress. Mild sedation and anti-inflammatory drugs can be effective in reducing pharyngeal swelling (Fossum, 2007; Meola, 2013).

Surgery Treatment

Early surgical intervention of brachycephalic airway syndrome pathologies is recommended to stop the progression of airway pathology (Trappler and Moore, 2011b; Dupré and Heidenreich, 2016). Surgical treatment has been found to be indicated, especially in the treatment of narrow nostrils and elongated soft palate. Recent studies have shown that in all brachiocephalic patients, clinical signs are significantly reduced in all brachiocephalic dogs of all ages and after surgery (Erjavec et al., 2021).

Preoperative Preparations

The operation should be prepared so that acute dyspnea can be followed and a tracheostomy can be performed urgently. Because the existing pathology and intraoperative intervention in the airway may cause postoperative edema in the airways in the postoperative period. (Trappler and Moore, 2011b). The patient should be carefully monitored in terms of decompensation and progressive respiratory distress, and tracheostomy should be applied when necessary. Perioperative IV corticosteroid therapy may help reduce airway swelling, but airway obstruction remains a potential complication during or after extubation (Fossum, 2007; Trappler and Moore, 2011b).

Anesthesia

In order to minimize the risks of anesthesia, it should be planned to prevent or correct complications that may occur during the perianesthetic period. There is no protocol applicable to all brachycephalics, it must be individualized and individualized for each patient according to the situation and the intervention. The purpose of premedication and induction in these breeds is to provide sedation without significant respiratory depression, reduce anxiety, reduce the required induction dose, prevent vomiting, and provide analgesia. Premedication may vary depending on the surgical intervention to be performed. It is recommended to use antiemetics (for example, maropitant and metoclopramide) especially in patients who will undergo soft palate surgery and in patients who vomit easily (Risco-López, 2015). Anticholinergics are never routinely used, only in cases of severe bradycardia during anesthesia (Bernaertsa et al., 2010).

The most commonly used alpha 2-agonists are medetomidine and dexmedetomidine. They are the best option in stable animals without cardiovascular disorders, as they provide very good sedation and analgesia as well as being reversible at low doses. Acepromazine should not be preferred because it causes relaxation of the pharynx muscles due to its peripheral vasodilator effect that increases heat loss. Benzodiazepines can be used but have little sedative power and should not be preferred as they can increase aggression. Although opioids produce bradycardia and respiratory depression at high doses, they show little adverse effect at usual doses. The choice depends on the type of intervention. The most commonly used opioid agents are methadone and fentanyl (Risco-López, 2015).

Preoxygenation is always very important before the operation in brachycephalic dogs. Because almost all drugs used in induction have a depressant effect on respiratory function (Koch et al., 2003). Propofol is the most used agent in induction because it provides rapid induction. In addition, respiratory depression that it may cause is rare. Alfaxalone causes little cardiovascular depression and may be preferred because it can be given intamuscular. Although ketamine causes little cardiovascular depression, it may support laryngospasm by not eliminating the reflex, so it may be preferred over operative intervention. Etomidate is indicated in cardiovascularly susceptible patients (Risco-López, 2015).

In brachycephalic breeds, we can use isoflurane or sevoflurane as inhalation anesthesia for the continuation of anesthesia, but care should be taken as these can suppress respiration. Injectable propofol or alfaxalone can also be used as the safest drug in these patients, but since they are breeds where oxygen supply is important, they should be intubated and given oxygen whether or not inhalation anesthesia is used for maintenance (Risco-López, 2015).

During the recovery period, the endotracheal tube should be kept in place until the swallowing reflex occurs to prevent aspiration pneumonia (Hobson, 1995). Before extubation, fluids such as mucus and blood in the upper respiratory tract should be checked. The patient should be placed in the sternal position with the head slightly elevated and the tongue extended. A material should be used to raise the chin. If sedatives are needed while waking up, low doses of alpha2-agonists, benzodiazepines or acepromazine can be used as sedatives (Risco-López, 2015).

Positioning

In operations to be performed inside the mouth, the patient is placed in a dorsoventral position with its mouth completely open. The maxilla should be suspended from a stick or two infusion carriers that are passed slightly above the operating table, and the mandible should be secured with tape ventrally. The tongue should be pulled rostrally for maximum visibility of the inside of the mouth (Trappler and Moore, 2011b). Modified apparatus made of stainless steel, consisting of a rectangular frame that opens the maxilla and mandible, can also be used (Kuraji et al., 2019).

As an alternative to the operations of the soft palate and laryngeal vesicles, the patient can be placed in the ventrodorsal lying position. In the operation performed for the stenotic nostril, the dorsoventral lying position can be performed with the mouth closed by supporting the chin. The patient's head should be taped to the table to prevent the head from turning (Fossum, 2007).

Surgical Techniques

In brachycephalic airway syndrome, different surgical techniques (eg, resection of stenotic nostrils, resection of everted laryngeal vesicles) have been described for most anatomical pathologies (Fossum, 2007; Hueber, 2008; Lippert et al., 2010).

Stenotic Nostril Resection (Rhinoplasty)

Various surgical techniques are used for resection of stenotic nostrils. All of the techniques used are used to widen the external nostrils (Fossum, 2007; Dupré and Heidenreich, 2016). Techniques such as alar amputation, wedge resection, punch alaplasty and alapexy are among the techniques that have positive results in the permanent widening of the stenotic nose (Aiken, 2021). It is recommended that the operation for stenotic nostrils, which is one of the upper respiratory tract pathologies, should be performed when the dog is 3 to 4 months old (Koch et al., 2003; Trappler and Moore, 2011b). In nostril resection, the number 11 scalpel is mostly preferred in order to make deep incisions. However, skin biopsy can also be performed with a punch tool, a fine-tipped electrosurgery or radiofrequency unit, or laser (Dupré et al., 2012).

Resection of Alae Nasi

Alar amputation involves resection of the soft tissue cranioventral wing to the dorsolateral nasal cartilage (Schlicksup, 2016). The incision line should be 15º downward and outward in the anterior view, and 40º downward and inward in the lateral view. Firm pressure is applied to the surgical site for 5 to 10 minutes to achieve hemostasis. The scar tissue formed after amputation may remain white for several months (Huck et al., 2008; Tobias, 2017).

Alaplasty

The most commonly used techniques are alaplasty techniques. It is made by removing a wedge from the ala nasi. This wedge can be vertical, horizontal or lateral (Dupré et al., 2012). Two to four simple separate sutures should be made using absorbable monofilament material for suturing. When the wound edges are sutured, it provides hemostasis (Dupré and Heidenreich, 2016).

Vertical Wedge Technique

In the vertical wedge technique, the ventral part of the alar fold of the stenotic nostril is held with Brown Adson forceps (Tobias, 2017). The first V-shaped incision should be made medially and the second incision should be made laterally with a scalpel around the forceps (Fossum, 2007). The lateral border of the wedge should be at an angle (40-70°) from the medial border. It is important that the removed tissues are deep enough and contain part of the alar fold to completely remove the obstruction. Care should be taken to achieve a symmetrical opening in both nostrils (Dupré et al., 2012).

Horizontal Wedge Technique

The horizontal wedge technique was initially described with various definitions. It was described as a right-angled wedge technique in which the tip of the wedge is positioned laterally (Harvey, 1982; Hobsan, 1995). According to the new definition, horizontal wedge resection involves creating a wedge from medial to lateral (Bofan et al., 2015).

Lateral Wedge Technique
The lateral wedge resection technique consists of excision of a vertical wedge of tissue from the caudolateral aspect of the outer nose, between the nose and the skin. The wedge may contain part of the skin (Nelson, 1993; Monnet, 2003) or not (Aron and Crowe, 1985). The wedge should be made deep to include part of the alar fold (Wykes, 1991).

Punch Resection Alaplasty
It is a technique in which a dermatological punch biopsy is used for the portion of the ala nasi to be resected (Trostel and Frankel, 2010). The diameter of the punch biopsy instrument used ranges from 2 mm for puppies to 3-6 mm for adult dogs (Dupré et al., 2012).

The alar fold is held for control and a dermatological punch tool is used to create a circular tissue symmetrical to the level of the alar fold at the ala nasi. The circular tissue is keeped using Thumb forceps and resected with tissue scissors after little traction. Bleeding is minimal at this stage. Pressure should be applied to stop bleeding or cotton swabs impregnated with epinephrine can be used (Trostel and Frankel, 2010).

Dorsal Offset Rhinoplasty
Dorsal offset rhinoplasty for use as the production of a rostral, most dorsal, wedge of each nasal planum and nasal cartilage. The width of the wedge should be approximately 3 to 5 mm, depending on the size of the animal and the severity of the stenosis. The first suture should be placed from the most rostral aspect of the axial edge to the most caudal aspect of the axial edge (Dickerson et al., 2020).

Alapexy
Alapexy is a method that can be used in dogs with excessive drooping of the ala nasi (Bofan et al., 2015). The length of the incision can vary from 0.5 to 1 cm, depending on the size of the dog, and should be about 3 mm wide. Another incision is made into the skin 3 to 5 mm lateral to the ala nasi, opposite the first incision. From the opposing incision surfaces, first the underlying incision edges and then the outer incision edges are joined with a simple separate suture with an absorbable 3-0 or 4-0 thread (Ellison, 2004).

Elongated Soft Palate Resection (Staphylectomy)
The purpose of resection of the elongated soft palate is to shorten the caudal part of the soft palate to prevent obstruction of the rima glottidis on inspiration (Dupré and Heidenreich, 2016). In normal animals, the caudal edge of the palate slightly overlaps the tip of the epiglottis (Tobias, 2017). Different markings along the rostrocaudal axis have been proposed for the soft palate (Monnet, 2013). In recent studies, it has been suggested that resection of the soft palate at the level of the cranial junction of the tonsillar crypts will not lead to nasal aspiration (Brdecka et al., 2008). Scalpel blade, scissors, monopolar electrocoagulation, carbon dioxide laser, diode laser or ligasure can be used for soft palate resection (Dupré and Heidenreich, 2016). The traditional technique is excision with a scalpel blade or Metzenbaum scissors and using simple, continuous suturing of the oropharyngeal, nasopharyngeal mucosa (Trappler and Moore, 2011b).

Laser Staphylectomy
The required length of the soft palate is marked by removing it from under the endotracheal tube. Fixation sutures are placed on both sides of the elongated soft palate, distal to the prescribed resection line, using a monofilament thread. The palate is pulled in the rostral direction with fixation sutures. Moist gauze is placed between the endotracheal tube and the palate and the prescribed resection line is marked from different areas. The incision is started from the lateral part of the resection line. In this way, the entire tissue is resected and the final appearance is achieved (Tobias, 2017).

Staphylectomy with the Cut and Sew Technique
Suspension sutures are placed on the edges of the soft palate with monofilament thread or hemostatic forceps. In order to view the ventral surface, the tip of the palate is pulled in the rostral direction with Allis tissue forceps or fixation sutures (Tobias, 2017). In order not to cause excessive mucosal swelling, the soft palate should be intervened as little as possible. After the proposed resection site has been marked, one-third to one-half of it is cut with curved Metzenbaum scissors or a scalpel. Starting from the border of the palate, the oropharyngeal and nasopharyngeal mucosa are sutured together with a simple continuous suture. In this way, the remaining soft palate is cut and sutured and the operation is terminated (Fossum, 2007).

Folded Flap Palatoplasty
Folded flap palatoplasty was developed to correct both excessive length and excessive thickness of the soft palate (Dupré and Heidenreich, 2016). In this technique, while the soft palate is thinned by cutting a part of the oropharyngeal mucosa and soft tissues, it is also shortened by folding it on itself (Monnet, 2013).

The caudal edge of the soft palate is grasped with forceps or fixation sutures and pulled rostrally. The intended incision area is marked with an electrocautery. The soft tissues under the cut part of the soft palate, the ventral mucosa of the soft palate, the palatinus muscles and part of the levator veli palatini muscle are excised. The caudal edge of the soft palate is retracted rostrally to the rostral edge of the trapezoidal incision (FIindjii and Dupré, 2008). A temporary permanent suture can be used to pull the soft palate rostrally. Thus, it provides a better visualization of the free edge of the soft palate before the palatopexy sutures are placed, allowing the operation to be performed easily (Sun et al., 2022). The soft palate is then terminated with a simple separate suture (FIindjii and Dupré, 2008).

Eversion of Laryngeal Saccules
Excision of everted laryngeal sacs is controversial, but eversion is known to cause rimal glottidis obstruction (Fawcett et al., 2018). Electrocautery, scissors, tonsil traps or laryngeal biopsy forceps can be used for excision of everted laryngeal saccules (Dupré and Heidenreich, 2016). While performing the operation, the least intervention should be done and unnecessary manipulations should be avoided. Unnecessary manipulations can cause local obstructive edema postoperatively (Fossum, 2007). It is recommended to use an endoscope or suitable magnification devices to perform a complete and safe resection of the operation. Removal of everted laryngeal sacculess is recommended only in cases where eversion is significantly caused by obstruction, according to recent studies (Dupré et al., 2012). Temporary tracheostomy is recommended by some investigators for easier operation in the operative field, but this can also be accomplished by temporary extubation or by pushing the endotracheal tube to one side. After the saccule is visualized, the saccule is

grasped with an Allis tissue forceps or hemostat and retracted rostrally and resected with an instrument such as a scalpel, Metzenbaum scissors, or electrocautery. Bleeding is usually minimal and is controlled with little pressure and the operation is completed (Trappler and Moore, 2011b). In one study, unilateral resection was performed, and it was observed that there was no improvement in the unresected area despite the treatment of the nostrils and soft palate (Dupré and Heidenreich, 2016).

Laryngeal Collapse

Partial laryngectomy in the surgical treatment of laryngeal collapse has a very high (50%) mortality rate. Therefore, it is not recommended. Laser-assisted partial arytenoidectomy recommended for the treatment of laryngeal paralysis may provide some relief, but is not routinely used and more research is needed. Alternatively, arytenoid lateralization is a viable option for dogs with adequate mineralization of their laryngeal cartilage. In cases where these surgical methods are not sufficient, tracheostomy can be performed (Dupré and Heidenreich, 2016).

As a result of the researches, it was understood that when the primary diseases (stenosis of the nostrils, long soft palate and eversion of the laryngeal vesicles) were corrected, there was improvement in laryngeal collapsed brachycephalic dogs. This situation has made laryngeal collapse surgery insignificant, especially (Dupré et al., 2012).

Postoperative Care and Complications

Postoperative care should be in the form of delayed extubation, analgesic protocol, nasal oxygen supplementation for up to 24 hours, and respiratory respiration monitoring (Koch et al., 2003). The endotracheal tube should not be removed during recovery as long as the patient tolerates it. Edema of structures in the airway, decreased pharyngeal reflexes, hyperthermia, and increased risk of aspiration are present for at least 24 to 48 hours after surgery. Therefore, the patient should be followed closely (Trappler and Moore, 2011b). Optimally, the animal should lie dorsoventrally as it awakens, with its front legs extended forward and outward. In addition, the head and neck should be extended and the tongue should be pulled forward to help open the airway (Fossum, 2007). Prednisone (0.5 to 1.0 mg/kg IV for 24 to 48 hours postoperatively) can be used to help reduce airway inflammation. Dogs should not be fed for 12 to 24 hours after the operation (Trappler and Moore, 2011b). Ice chips can be given when fully awakened from anesthesia, but no food should be given. Water must be given first; If tolerated well, small amounts of soft food can be offered (Fossum, 2007). The diet should consist of only soft foods for 10 to 14 days to minimize irritation of the upper respiratory tract. During this period, patients should be kept in a cool environment, moderately active, and kept the pet in poor body condition. To relieve the stress on the upper respiratory tract of dogs, a body collar should be used, not a neck collar. These lifestyle changes must be sustained throughout the patient's life to maximize successful outcomes (Trappler and Moore, 2011b).

Serious postoperative complications include airway swelling, vomiting, and aspiration (Trappler and Moore, 2011b). The mortality rate after staphylectomy is below 5%. If palate resection is inadequate, clinical signs will likely reappear. If the resection is excessive, the animal will reflux water and food through its nose and may develop cough and rhinitis (Tobias, 2017). If the patient frequently licks or rubs her nose, a pink scar may remain (Fossum, 2007).

CONCLUSION

Brachycephalic dog breeds have been around for over 2000 years. Recently, as a result of the increase in brachycephalic dog breeds and population, the diseases in this breed have been brought to the agenda more. It is known that especially primary upper airway diseases seen in brachycephalic dogs adversely affect the quality of life of these dogs. In addition to many visual diagnosis methods used in the diagnosis of primary upper airway diseases, clinical appearance, physical examination findings and laboratory findings are also important. In the treatment of brachycephalic upper airway syndrome, especially surgical treatments are of great importance following medical treatments.

Conflict of Interest

The authors declared that there is no conflict of interest.

Authorship contributions

Concept: G.D., R.Y., Design: G.D., R.Y., Literature Search: G.D., R.Y., Writing: G.D., R.Y.

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REVIEW ARTICLE

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Fundamental Molecules in the Pathways and Regulation of Apoptosis

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Abstract

Apoptosis plays a role in many events such as the remodeling of cells starting from embryo formation, the elimination of faulty or dysfunctional cells, and is important for cellular balance. Bcl-2 family and various mediators, of which caspases are the basis, play a role in maintaining this balance in apoptosis. These mediators are important in the initiation, continuation and arrest of apoptosis pathways, and in the regulation of cellular balance. Damages to these mechanisms can cause undesirable results and diseases. In this review, we tried to give information about the pathways of apoptosis and the basic molecules involved in the regulation of apoptosis.

Keywords: Apoptosis, Bcl-2, caspase, pathways, regulation.

INTRODUCTION

Apoptosis occurs throughout life, starting from the embryonic period. It is a programmed cell death that is genetically regulated in eliminating unnecessary tissues, reorganizing them, eliminating cells that have completed their life, and maintaining intracellular balance (Khan et al., 2010). It consists of the words 'apo' and 'ptosis', which are used to mean separate and fall. It is an energy requiring process (Karalezli, 2016). First, in Kerr et al., (1972) observed condensed chromatin fragments in the nuclei of cells that died physiologically in their microscopic examination and, realizing that the organelles were preserved, they defined this phenomenon as "shrinkage necrosis". While *Caenorhabditis elegans* nematode, which is frequently used in apoptosis studies, passed from the hermaphrodite period to the adult stage, a decrease of 131 cells was determined in the number of cells that apoptosis result was 1090 (Hengartner and Horvitz, 1994). The cell death abnormal-3 (ced-3), cell death abnormal-4 (ced-4), and cell death abnormal-9 (ced-9) genes are involved in the regulation of apoptosis in these nematodes. It was observed that when ced-3 and ced-4 of these genes are inactivated, apoptosis is not observed in cells and these genes are the genes that stimulate apoptosis, while the cell death abnormal-9 (ced-9) gene is the gene that suppresses apoptosis. These genes have been named as caspases (Cysteine aspartate-specific proteases), Apaf-1 (Apoptotic protease activating factor) and Bcl-2 (B-cell lymphoma protein-2) accordingly in humans (Hengartner, 1996).

MORPHOLOGICAL CHANGES OBSERVED IN APOPTOSIS

The main structural changes observed in apoptosis are cell shrinkage, chromatin condensation and subsequent fragmentation. This fragmentation, which causes the DNA to show blebs in fluorescent staining, occurs through the enzyme endonuclease, and DNA is fragmented from its internucleosomal domains. When immune electrophoresis is performed, a ladder-like structure called 'ladder pattern' is observe (Wyllie, 1980). Budding forms on the cell membrane of apoptotic cells and the cell fragments into apoptotic bodies (Figure 1) (Cotter et al., 1992). These cells are then phagocytosed by macrophages and neighboring cells without the occurrence of inflammation (Kerr et al., 1972).

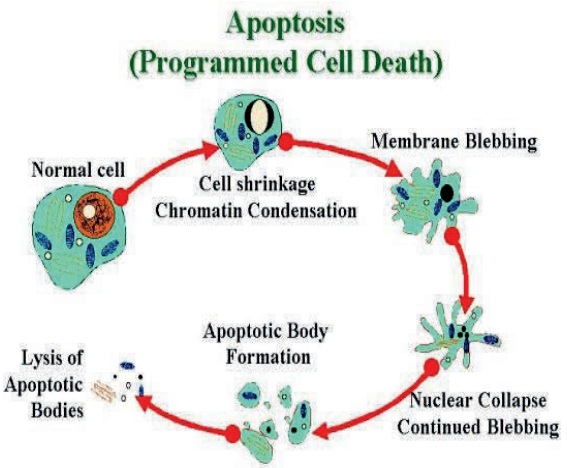

Figure 1. A schematic of a cell in apoptosis (https://www.quora.com/Why-do-cells-undergo-apoptosis)

MECHANISMS OF APOPTOSIS

Extrinsic and intrinsic pathways constitute the basis of apoptosis. Proteases called caspases are involved in these pathways, and both pathways enter a common pathway with the conversion of procaspase-3 to its active form. In addition to these pathways, there is also the

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perforin/granzyme pathway in which cytotoxic T cells and perforin/granzymes are involved (Elmore, 2007).

Extrinsic Pathway

Transmembrane receptors, which are included in the TNF (tumor necrosis factor) receptor family, are involved in this pathway. These receptors have a death domain containing almost 80 amino acids in their structure responsible for signal transmission (Park et al., 2014). Events occurring in the extrinsic pathway are schematized by FasL/FasR (Fas ligand/Fas Receptor) and TNF-α/TNFR1. The interaction of the Fas receptor with its ligand provides binding to the death domain FADD (Fas associated death domain) which the Fas receptor is associated. The interaction of the TNF receptor with its related ligand allows the TNF receptor to bind to TRADD (TNFR-1-related death domain) via FADD and RIP (receptor interacting protein). Subsequently, FADD dimerizes and can thus bind to procaspase-8. This binding causes the formation of the DISC complex (death inducing signaling complex) and the conversion of procaspase-8 to the caspase-8 form by autocatalytic activation (Falschlehner et al., 2007). If active caspase-8 is at high levels, it is degraded to terminator caspases such as caspase-3 as a result of autoproteolytic cleavage, or activated caspase-8 is triggered by caspase-8 signaling to break Bcl-2 family members Bid and release cytochrome-c from mitochondria to stimulate apoptosis. Depending on this, it also mediates the realization of events that result in caspase-3 activation (Mayer and Oberbauer, 2003). This pathway can be suppressed by a protein called c-FLIP (cellular FLICE-like inhibitory protein) (Golks et al., 2005).

Intrinsic Pathway

Instead of receptors, many stimuli such as growth factor deficiency, oxygen deficiency, high temperature, viral factors play a role in the initiation of this pathway. These stimuli cause Bcl-2 family member and apoptosis-stimulating proteins to migrate to mitochondria and attach to the mitochondrial outer membrane, resulting in the formation of the mitochondrial transition pore (Giorgi et al., 2012). This pore is involved in the release of cytochrome-c from the mitochondria to the cytosol (Giorgi et al., 2012).

Cytochrome-c then forms a complex called apoptosome with Apaf-1, ATP and procaspase-9. The apoptosome complex is involved in the transition of procaspase-9 to its active caspase-9 form (Elumalai et al., 2012). The conversion of procaspase-9 to its active form can be inhibited by a protein called Aven (Chau et al., 2000). Activated caspase-9 causes caspase-3 activation as at the end of the extrinsic pathway. Proteins such as AIF (apoptosis-inducing factor), endonuclease G and Smac/DIABLO and Omi/HtrA2, which translocate to the nucleus and cause DNA segmentation and chromatin condensation, are also released from the mitochondria and neutralize the effect of IAPs (apoptosis-inhibiting protein) on apoptosis (Savitskaya and Onishchenko, 2015).

Perforin/Granzyme Pathway

In this pathway, apoptosis is induced via GranzymeA or GranzymeB. Caspase-3 activation is observed in all three of the extrinsic, intrinsic and GranzymeB pathways. Cytotoxic T cells and natural killer (NK) cells contain perforin and granzyme proteins in their secretory granules. Secreted perforins cause pore formation in the cell after the interaction of cytotoxic T cells with the target cell (Turner et al., 2019). While Granzyme B enables the conversion of caspase-3 to the active form, either directly or by the activation of procaspase-10; granzyme A stimulates the cell to enter the apoptosis pathway by single-stranded DNA damage. (Martinvalet et al., 2005).

Path of Execution

It begins with the activation of terminator caspases to the active form in the final stage of the extrinsic and intrinsic pathways. Terminator caspases are involved in the activation of endonucleases, which cause nuclear material fragmentation, and proteases responsible for cytoskeletal proteins and nuclei fragmentation. Caspase-3, has an important role in apoptosis. Caspase-3, leads to the activation of the endonuclease CAD (caspase-activated DNase) and cleaves the CAD inhibitor, ICAD, leaving CAD free for DNA fragmentation and chromatin condensation and apoptosis is terminated by phagocytosis of apoptotic cells (Larsen and Sørensen, 2017).

REGULATION OF APOPTOSIS

Regulation of the apoptotic process is provided by many molecules. Bcl-2 family proteins, caspases, mitochondria, cytochrome-c, calcium are among the molecules responsible for regulation (Akşit et al.,2008)

Calcium

During apoptosis, the cell is exposed to a constant flow of calcium. As a result of the increase in intracellular Ca^{+2} concentration, endonucleases are activated and this causes the initiation of the cell death cascade. The increase in calcium concentration occurs with the increase of Ca^{+2} influx from Ca^{+2} channels and Ca^{+2} release from organelles such as mitochondria and ER (Sukumaran et al., 2021).

Bcl-2 Family

There are antiapoptotic and proapoptotic members of this protein family that suppress and stimulate apoptosis. Although the Bcl-2 family does not differ much in structure, they have similar protein structures with α helix tangles. These are called BH domains and are numbered up to 4. In addition, they are examined in 3 main groups (Gross et al., 1999).

Group I: Proteins in this group are located in the mitochondrial outer membrane, nuclear membrane, and endoplasmic reticulum and are involved in the suppression of apoptosis by inhibiting the release of procaspase, AIF and cytochrome-c. Bcl-2, Bcl-xL, Mcl-1, Bcl-w, Bfl-1/A1, boo proteins constitute the members of this group (Gross et al., 1999).

Group II: Mostly containing 3 sometimes 2 BH domains, these group members are Bax, Bak, Bcl-Xs. They are present in the cytoplasm and, unlike group I, they stimulate apoptosis by positively affecting the release of AIF and cytochrome-c (Gross et al., 1999).

Group III: Among the group members are BH3 interacting-domain death agonist (Bid), Bcl-2-interacting killer (Bik), Bcl-2-interacting mediator of cell death (Bim), Bcl-2-associated death promoter (Bad), p53 upregulated modulator of apoptosis (Puma), NADPH oxidase activator (Noxa), which contain only the BH3 domain and are associated with the mitochondrial outer membrane. Members of this group connect both with each other and with mitochondrial membrane proteins. They

play a role in the release of cytochrome-c into the cytosol by forming a pore in the mitochondrial membrane. Proteins in this group do not exist in the active form and after activation, they allow the activation of group II proteins (Gross et al., 1999).

Caspases

Cysteine aspartate-specific proteases are released in the form of procaspase and are activated by cleavage of the protein from the aspartate amino acid after stimulating apoptosis. All caspases contain catalytic small and large subunits in their structure. It is associated with the length and shortness of the N-terminal front region found in the classification structures of caspases classified as initiator and terminator. A long N-terminal anterior region is observed in initiator caspases, while a short N-terminal anterior region is observed in terminator caspases (Stennicke and Salvesen, 2000).

Caspase 3-6-7-14 terminator, caspase 1-2-4-5-8-9-10-11-12-13 are initiator caspases and initiator caspases contain a CARD (caspase-recruitment domain) or DED (death effector domain) domain in the long N terminal region. Of these caspases, caspase-8 and caspase-10 have a DED domain, while other initiator caspases have a CARD domain. Terminator caspases do not have CARD and DED domains (Ramirez and Salvesen, 2018). Caspase-8, 9, 10 causes caspase-3 activation and initiation of the execution phase (Ramirez and Salvesen, 2018).

Inhibitors such as XIAP (X-linked apoptosis protein inhibitor) and IAPs can prevent caspase-3 activation (Nachmias et al., 2004). In addition, caspase-1, 4, 5, 11, 12, 14 plays different roles such as inflammation and keratinocyte maturation. Additionally, caspase-2 specifically reverts to its active form with the PIDDosome complex. This complex consists of PIDD, RAIDD and caspase-2 and is stimulated by p53 as a result of DNA damage (Parrish et al., 2013).

p53

The p53 gene, which is responsible for the regulation of apoptosis and tumor suppression as a result of oxygen deficiency and the formation of free radicals, provides the necessary time for DNA repair by preventing the cell from transitioning to S phase when DNA damage occurs. If there is a damage that cannot be repaired, it stimulates apoptosis by increasing the production of Fas, Bax and Apaf-1 and inhibiting Bcl-2, Bcl-xL. The p53 gene is suppressed by the transcription regulator factor Mdm-2 (murine double minute2) (Vousden and Lu, 2002).

Fas/Apo-1/CD95

Fas receptor, which is a member of TNF receptor family and located on the surface of natural killer and cytotoxic T cells, is activated as a result of its association with Fas protein, and when it binds to the FADD molecule, procaspases turn into active form and apoptosis is stimulated (Volpe et al., 2016).

Apaf-1

It is a protein that plays a role in the initiation of apoptosis and has CARD and ATPase regions in its structure. After cytochrome-c is released into the cytoplasm in the intrinsic pathway, its structure changes as a result of the interaction of cytochrome-c with the CARD domain. And through the CARD domain, Apaf-1 and caspase-9 interact to form apoptosome complexes. This complex is involved in the transition of effector caspases to the active form (Droin and Green, 2004).

Cytochrome-c

It is an important protein in the intermembrane space of mitochondria and is involved in the production of ATP. Observation of cytochrome-c in the cytoplasm indicates that the cell is in an irreversible process of apoptosis. Although the transition of cytochrome-c to the mitochondrial outer membrane remains unclear, some members of the Bcl-2 family are involved in the regulation of this process (Gross et al., 1999).

RESULT

Many molecules are involved in apoptosis, which play a role in the elimination of unwanted, expired cells, mediating the ability of cells to perform their vital activities, and many pathways are observed for the continuation of the apoptosis process. In this review, we have mentioned the main molecules involved in the pathways and regulation of apoptosis. We think that as a result of the studies, more molecules will be discovered and the mechanisms will be understood more clearly, so that it will be a guide in terms of the occurrence and treatment of many diseases.

Conflict of Interest

The authors declared that there is no conflict of interest.

Authorship contributions

Concept: S.T., Design: S.T., B.O., Data Collection or Processing: S.T., B.O., Analysis or Interpretation: S.T., B.O., Literature Search: B.O., Writing: S.T., B.O.

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