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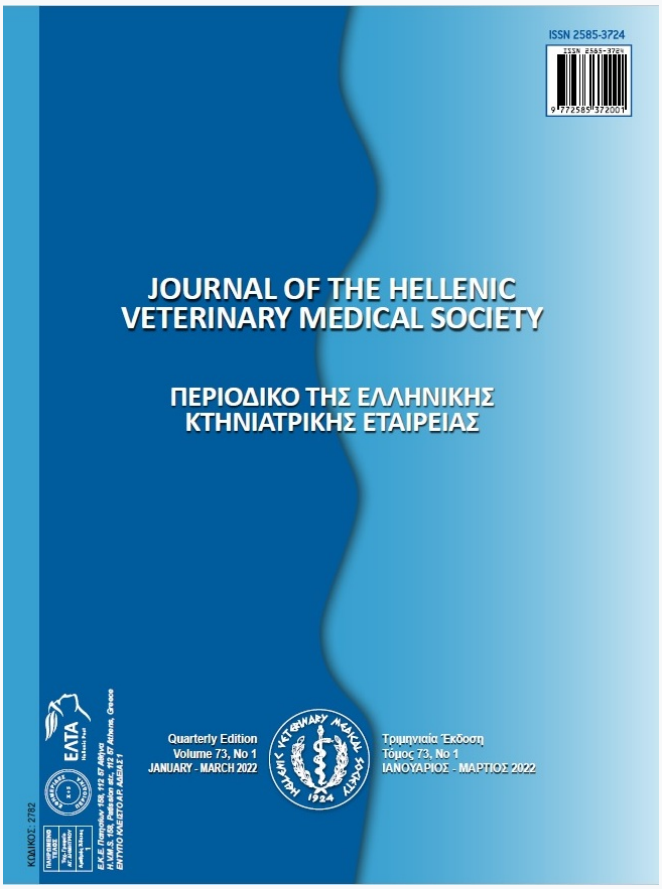

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Research article
Ερευνητικό άρθρο

Ketamine or propofol anesthesia in dogs: how do they affect cytokines, antioxidants and neutrophil functions?

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ABSTRACT: The objective of the study is to investigate the effects of ketamine and propofol on cytokines, antioxidant defense system, and neutrophil functions in dogs. A total of 24 dogs were used. Dogs were divided into two groups as ketamine and propofol. The ketamine group received ketamine (5 mg/kg) intravenously while the propofol group received propofol (4 mg/kg) intravenously.Blood samples were collected before sedation and 30 minutes after induction of anesthesia.Serum antioxidant and cytokine levels were analyzed and neutrophil functions were determined. Respiration rate, serum malondialdehyde, IL-4, IL-6 levels, and phagocytic and chemotaxic activity of neutrophils were decreased (P=0.001, P=0.010, P=0.014, P=0.039, P=0.008, and P=0.037, respectively), oxygen saturation were increased (P=0.025) in the ketamine group. Serum IL-6 and IFN-γ level were decreased (P=0.015 and P=0.032 respectively), chemotactic activity of neutrophils were increased (P=0.049) in propofol group. The administration of ketamine was found to have a positive effect both on the antioxidant system and the neutrophil. On the other hand, positive and negative effects of propofol on different parts of the immune system were observed. Therefore, the results should be taken into account when designing an anesthesia protocol for dogs to predict possible defense system reactions during the postoperative period.

Keywords: Dog, anesthesia, cytokine, antioxidant, neutrophil activation

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INTRODUCTION

Today, it is not enough for anesthetic drugs to provide only an effective anesthesia. In addition, evaluation of the potential effects on the immune system, defense mechanisms and cytokines is important to minimize possible postoperative complications (Soneja et al., 2005). Ketamine, which is frequently used in veterinary practice, is a dissociative anesthetic and is preferred due to its non-suppressive effects on the cardiorespiratory system in clinical doses (Muir, 2010). Propofol is commonly used for induction and maintenance of anesthesia (Jungheinrich et al., 2002).

Antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) play a protective role against oxidative stress (Szczubial et al., 2015). Ketamine exhibits antioxidant features in experimental studies in animals (Wang et al., 2019). However, the reported cases are quite limited in dogs (Camkerten et al., 2016). Propofol, which is structurally similar to antioxidants, is reported to be protective against oxidative stress by increasing SOD, CAT and GSH-Px activities in dogs (DeLa Cruzet al., 1999; Lee and Kim, 2012).

Cytokines are small proteins involved in generation of an immune response and inflammatory reactions. Anesthetics might have had an impact on cytokine production and proinflammatory-antiinflammatory cytokine balance (Sheeran and Hall, 1997). Human research revealed the suppressive effects of ketamine (Lisowska et al., 2013) and propofol (Chen et al., 2005) on proinflammatory cytokines. However, studies in dogs are very limited. Therefore, the possible effects of anesthetics on cytokines are considered worthy of investigation.

Neutrophils, phagocytic cells, are the first line of cellular defence against pathogens. It is not sufficient to merely estimate the count of neutrophils in the peripheral circulation to evaluate their effectiveness. Therefore, their phagocytic and chemotactic activities and as well as oxidative burst should also be explored because the involved mechanisms play an important role in generating an immune response by sending signals to other cells of the defence system (Dinauer, 2007; Van Kessel et al., 2014). Anesthetics agents are known to affect the circulatory neutrophil count and chemotaxis (Morisaki et al., 1998) but their effects on neutrophils are not limited to the relevant events. Propofol and ketamine were indicated to suppress phagocytic activity, oxidative burst, and chemotactic activity in humans (Nishinaet al., 1998; Cruz et al., 2017). Particularly, propofol inhibited superoxide production by neutrophils in dogs (Sato et al., 2016), while inhibition of phagocytic response by polymorphonuclear leukocytes for ketamine was shown in vitro animal studies (Son et al., 2009), but no clinical study was referred.

Ketamine and propofol affect the body's defence and antioxidant systems. Although cytokines play a substantial role in the relevant interactions, this mechanism has not been thoroughly investigated, considering species-related differences in animals. The aim of this study is to investigate the effects of ketamine and propofol on cytokines, antioxidants and neutrophil functions just 30 minutes after their administration for anesthesia induction in dogs premedicate with alfa 2- adrenergic agonists. It was hypothesized that ketamine or propofol, used as anesthesia agents in dogs, will affect inflammatory response, antioxidant defence mechanism and neutrophils in a different way. This was thought to have important effects on the patient's recovery from anesthesia period.

MATERIALS AND METHODS

The study was approved by the Istanbul University Experimental Animals Local Ethics Committee (no. 35980450-050.01.04) with informed owner consent.

Animals

A total of 24 healthy, male dogs aged 1to6 years old, ASA I and II, were enrolled in the study (Table 1). The mean body weight (± standard deviation) was 22.17 ± 13.02 kg. Physical examination, complete blood count, and biochemical parameters were applied on each animal. Food and water intake were withheld for 8 hours and 1 hour, respectively, before anesthesia.

Experimental design

The article was prepared according to the guidelines proposed by the CONSORT (Moheret al., 2010) (Figure 1). The dogs were randomly allocated into two groups (n = 12) "ketamine" or "propofol" group. A catheter was inserted into the cephalic vein for intravenous injections. For premedication, xylazine (ROMPUN, BAYER) at 0.5 mg/kg was administered intravenously (IV) to all dogs. Five minutes after premedication, dogs in the ketamine group received ketamine (ALFAMINE, EGE-VET) at a dose of 5 mg/kg IV, while the animals in propofol group received 4 mg/kg propofol (PROPOFOL, FRESENIUS) IV. Endotracheal intubation was performed a cuffed tube

Table 1. The breed and age distribution of the ketamine and propofol groups and the reasons for the anesthesia of the cases

	Ketamine (n = 12)	Propofol (n = 12)
Breed		
Golden Retriever	2	1
English Cocker Spaniel	1	0
Cross Breed	3	2
Anatolian Sheepdog	1	1
English Setter	1	2
French Bulldog	1	1
German Shepherd Dog	1	0
Chow Chow	1	1
Rottweiler	1	0
Terrier	0	2
Pug	0	1
Jack Russell Terrier	0	1
Age (years)		
1	4	4
2	1	0
3	3	2
4	3	3
5	0	0
6	1	3
Reasons for anesthesia		
Osteosynthesis	7	8
Soft tissue surgery	5	4

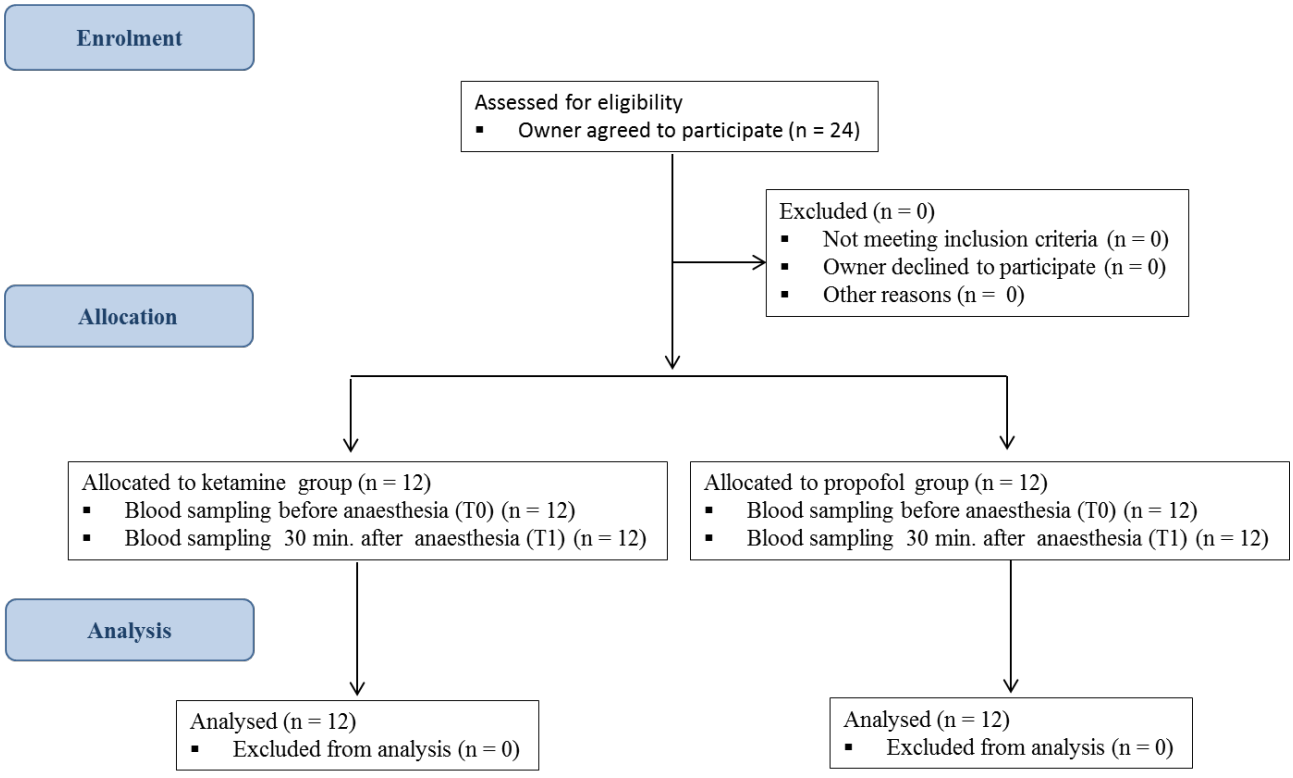

Figure 1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram

(WILLY RÜSCH) and anesthesia was maintained with isoflurane at a concentration of 2% (vaporizer setting) in 100% oxygen in spontaneous breathing. All dogs received Lactate Ringer's solution (10 ml/kg/hr, IV) during anesthesia.

Monitoring of cardiorespiratory parameters

Heart rate (HR), respiration rate (RR), end-tidal carbon dioxide ($ETCO_2$), hemoglobin oxygen saturation (SpO_2) and rectal temperatures (RT) of all cases were monitored throughout the anesthesia by a multifunctional ECG monitor (Advisor V9212 AR, SURGIVET). Data were recorded every 5 minutes, before sedation (T0) and at 30 minutes after anesthesia induction (T1) and before the surgical incision were assessed.

Blood sampling

Blood samples, which were taken from jugular vein at times T0 and T1, were collected into both serum and heparinized tubes, each containing 3 ml of blood. Heparin-containing whole blood samples were used to assess neutrophil functions while anticoagulant free blood specimens were centrifuged at 3000 g to obtain serum samples. The samples were stored at -80 ºC for further analysis.

Analysis of oxidative stress parameters

Serum MDA level was measured in all cases to assess oxidative stress that ketamine and propofol anesthesia might have induced. Serum SOD, CAT, and GSH-Px levels were measured to determine the efficacy of the antioxidant defence system. Analyses of MDA and antioxidant enzymes were performed by the ELISA method using dog-specific commercial kits (ABBKINE, ABBKINE SCIENTIFIC) and following the manufacturer's instructions. Serum samples were used to detect changes in the antioxidant defence system.

Plasma thiobarbituric acid reactive substances (TBARS) were estimated according to the method of Yoshoiko et al. (1979). The assay was based on the reaction of two molecules of thiobarbituric acid with one molecule malondialdehyde. This formed a coloured complex with a maximum absorbance at 532 nm. Plasma Cu-Zn superoxide dismutase SOD activity was determined according to method of Sun et al. (1988) by inhibition of nitroblue tetrazolium (NBT) reduction with xanthine-xanthine oxidase used as a superoxide generator. One unit of SOD is defined as the amount of protein that inhibits the rate of NBT reduction by 50%. CAT activity was determined by modified method described by Yasmineh et al. (1995). The assay was based on the decomposition of H_2O_2 in buffer by catalase enzyme in the plasma. GSH-Px activity was measured using spectrophotometrickits in accordance with the manufacturer's instructions. This method is based on that of Paglia and Valentine (1967).Glutathione Peroxidase (GPX) catalyses the oxidation of Glutathione (GSH) by Cumene Hydroperoxide. In the presence of Glutathione Reductase (GR) and NADPH the oxidised Glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP+. The decrease in absorbance at 340 nm is measured.

Assessment of cytokine level

Interlukin-2, IL-6, IL-8, IFN-γ levels, and IL-4, IL-10 ve TGF-β levels were measured to assess the possible effects of anesthesia on pro-inflammatory and anti-inflammatory cytokines, respectively, by the ELISA method using dog-specific commercial kits (ABBKINE, ABBKINE SCIENTIFIC) according to the manufacturer's instructions. Serum samples were used to detect cytokine levels.

Isolation of neutrophils and assessment of neutrophil functions

Three millilitres heparinized blood was layered carefully onto same amount of the Histopaque density gradient 1.5 ml Histopaque-1077 was layered onto 1.5 ml Histopaque-1119, (HISTOPAQUE, SIGMA-ALDRICH). The samples were then centrifuged at 340 g for 30 minutes at room temperature. After centrifugation the supernatant was discarded, and neutrophil containing Histopaque-1119 band was collected carefully by Pasteur pipet and transferred to a conical tube. Obtained cells were washed with 6 ml Hank's balanced salt solution (HBSS) three times (10 minutes at room temperature, 300 g) (Strasseret al., 1998). The cell pellets were re-suspended in 1 ml PBS and investigated for cell viability and measurement of neutrophil function. Cell numbers were adjusted to 1×10^6 cell/ml. Cell viability was determined by flow cytometer using rhodamine-123 (Robinsonet al., 1997).

To assess phagocytic activity, oxidative burst, and chemotactic activity, 20 µl of cell suspension was pipetted into 3 tubes that were added 1 ml PBS, 10 µldihydrorhodamine-123 (DHR-123, SIGMA-ALDRICH) and incubated in a 37 ºC water bath for 5 minutes. After incubation, 20 µl of E. coli solution (5

x 10^9 E. coli/ml), 10 µl (20 mg/ml DMSO) phorbol 12-myristate 13-acetate (PMA, SIGMA-ALDRICH), and 10 µl (4 mg/ml DMSO) N-Formylmethionyl-leucyl-phenylalanine (fMLP, SIGMA-ALDRICH) were added to the first, second and the third tube, respectively, to provoke phagocytic activity, oxidative burst, and chemotactic activity. After the stimulants were added (minute 0), the cell suspension was analyzed in flow cytometry. Mean fluorescence intensity was measured using the FL1 detector. After the first measurement, the cell suspension was incubated for 20 minutes in the water bath (37 ºC) and afterward, the mean fluorescence intensity was measured again. Phagocytic activity, oxidative burst, and chemotactic activity was calculated as the ratio of MFI value at 20 minutes. MFI-1 value at 0. minute (Bilgic et al., 2008). Flow cytometric analyses were performed on a flow cytometry equipped with cell quest software (FACS calibur, BD BIOSCIENCES).

Statistics

All statistical analysis were performed with IBM SPSS Statistics Version 21 program (SPSS Inc., CHICAGO, ILLINOIS). First, Shapiro-Wilk test was used to check whether the data obtained was normally distributed. The presence of a difference between the data obtained at T0 and T1 was analysed using paired samples t-test for normally distributed data. Non-parametric Wilcoxon signed rank test was used when the normality assumption was violated. The results are expressed as means ± standard error of the mean (SEM) for parametric variables and median with interquartile range (IQR) for nonparametric variables. Significance was established at $p < 0.05$ level.

RESULTS

The effects of ketamine or propofol on certain physiological parameters are shown in Table 2. No significant change was recorded in the ketamine group concerning HR and RT (p=0.352 and p=0.711) compared to the values before anesthesia. However, RR was significantly decreased (p=0.001) while SpO_2 was increased (p=0.025) at T1. No significant change was noted in the dogs that received propofol regarding HR, SpO_2, and RT (p=0.069, p=0.243, and p=0206, respectively) while RR was significantly decreased (p=0.012) at T1 compared to before anesthesia. Mean values of $ETCO_2$ levels that were measured after anaesthesia, were43.1 ± 7.02 and 41.3 ± 9.7 in ketamine and propofol groups, respectively.

The effects of ketamine or propofol on the antioxidant defense system are presented in Table 3.It was found that serum MDA level decreased significantly 30 minutes after ketamine administration, but there was no significant change in serum CAT, SOD and GSH-Px levels (p=0.540, p=0.689, and p=0.656, respectively). On the other hand, there was no significant change in serum MDA (p=0.273) or SOD, CAT, and GSH-Px levels (p=0.699, p=0.278, and p=0.255) in dogs anesthetized with propofol.

Table 2. Heart rate (HR, beats per min), respiratory rate (RR, breaths per min), heamoglobin oxygen saturation (% SpO2) and rectal temperature (RT, °C) measured before sedation (T0) and 30 minute after anesthesia induction (T1) in dogs anesthetized with ketamine or propofol

		HR (bpm)		RR (bpm)		SpO_2 (%)		RT (°C)	
		Mean ± SEM		Mean ± SEM		Median	(IQR)	Median	(IQR)
Ketamine	T0	107.0 ± 8.55		81.71 ± 7.13		90	(89-92)	38.7	(38.3- 39.1)
	T1	89.8 ± 12.93		10.21 ± 2.89		94	(93-95)	39.0	(38.1-39.5)
	P values	0.352		0.001		0.025		0.711	
		Median	(IQR)	Median	(IQR)	Mean ± SEM		Mean ± SEM	
Propofol	T0	115.0	(100-128)	55	(24-120)	92.5 ± 0.98		38.9 ± 0.11	
	T1	85.5	(72-129)	12	(10-16)	94.1 ± 0.82		38.7 ± 0.17	
	P values	0.069		0.012		0.243		0.206	

T0 = before sedation, T1 = 30 minutes after anesthesia induction. SEM= Standard error of mean, IQR = Interquartile range. Normally distributed data were presented as mean ± SEM, non-normally distributed data were presented as median and interquartile range (IQR). n = 12 in ketamine and propofol groups.

Table 3. Serum malondialdehide (MDA), catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) levels measured before sedation (T0) and 30 minute after anesthesia induction (T1) in dogs anesthetized with ketamine or propofol

		MDA (mmol/ml)		SOD (ng/ml)		CAT (ng/ml)		GSH-Px (ng/ml)	
		Mean	SEM	Median	(IQR)	Mean	SEM	Mean	SEM
Ketamine	T0	6.06 ±	0.61	5.66	(5.2-6.3)	53.7 ±	3.93	49.15 ±	4.44
	T1	5.35 ±	0.41	491	(4.4-6.1)	52.4 ±	3.32	46.19 ±	5.76
	P values	0.010		0.540		0.689		0.656	
		Mean	SEM	Median	(IQR)	Median	(IQR)	Mean	SEM
Propofol	T0	5.02 ±	0.33	6.8	(5.5- 7.2)	59.23	(55.5-104.3)	52.46 ±	5.65
	T1	5.60 ±	0.38	6.3	(5.3-10.8)	65.29	(59.0-92.8)	48.37 ±	4.45
	P values	0.273		0.699		0.278		0.225	

MDA = malondialdehide, CAT = catalase, SOD = superoxide dismutase, and GSH-Px = glutathione peroxidase, T0 = before sedation, T1 = 30 minutes after anesthesia induction. Normally distributed data were presented as mean ± SEM, non-normally distributed data were presented as median and interquartile range (IQR). n = 12 in ketamine and propofol groups.

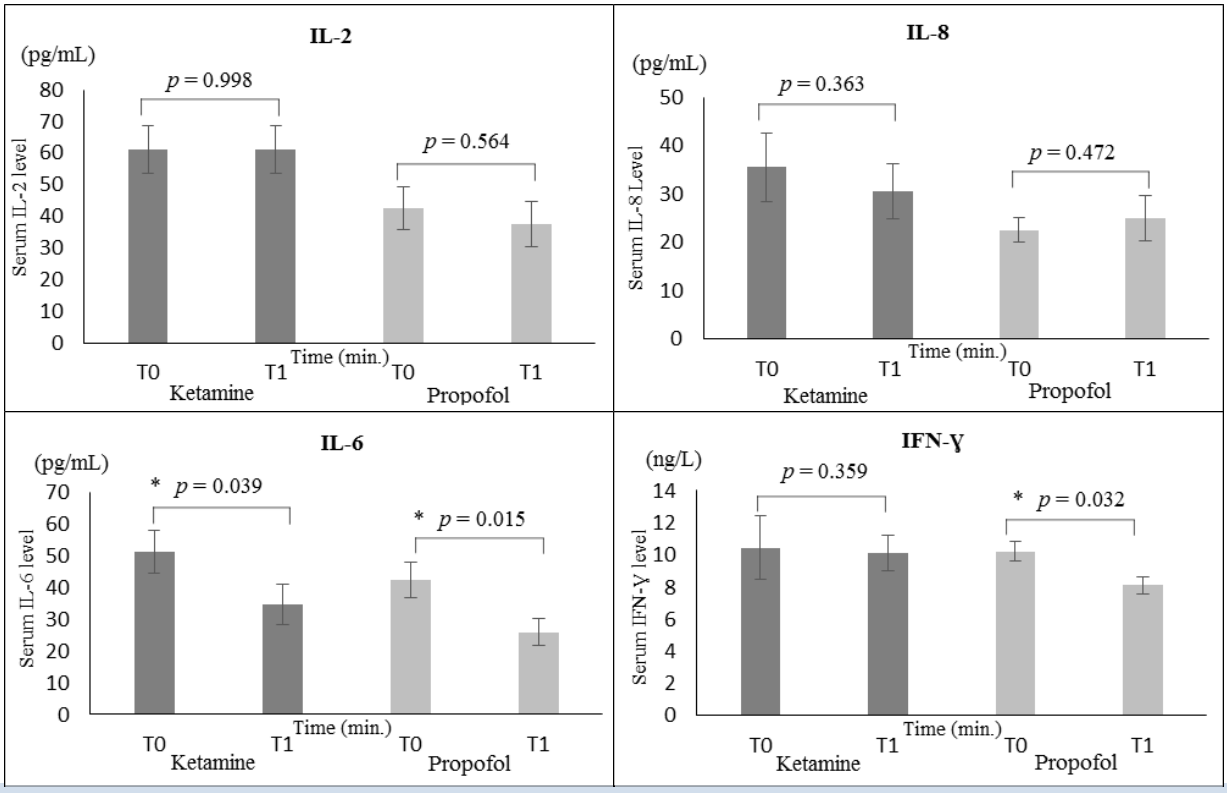

Figure 2. Mean values of interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-8 (IL-8) and interferon gamma (IFN-γ) of dogs in ketamine and propofol groups; before sedation (T0) and 30 minutes after anesthesia induction (T1). The error bars show the standard error of mean (SEM). * Indicates that the difference between T0 and T1 is significant. n = 12 in ketamine and propofol groups

Findings regarding pro-inflammatory cytokines are given in Figure 2. Thirty minutes after ketamine administration, serum IL-6 levels were significantly decreased compared to the value before anesthesia (p=0.039), but serum IL-2, IL-8 and IFN-γ levels did not change (p=0.988, p=0.363, and p=0.359, respectively). Propofol had no significant effect on serum IL-2 and IL-8 levels (p=0.564 and p=0.472, respectively) while IL-6 and IFN-γ levels were decreased (p=0.015 and p=0.032, respectively) compared to them before anesthesia.

Data regarding anti-inflammatory cytokines are shown in Figure 3. Serum IL-4 level in the dogs that received ketamine was significantly decreased (p=0.014) However, IL-10 and TGF-β remained un-

changed compared to the values before anesthesia (p=0.833 and p=0.688, respectively). In the propofol group, serum IL-4, IL-10, and TGF-β levels were not significantly affected compared to the values before anesthesia (p=0.432, p=0.470, and p=0.358, respectively).

The effects of ketamine or propofol on neutrophil functions were presented in Figure 4. It was determined that the phagocytic and chemotaxic activity of neutrophils decreased 30 minutes after ketamine administration (p=0.008 and p=0.037, respectively), however, the oxidative burst was not affected (p=0.419) at T1. Propofol did not alter the phagocytic activity and the oxidative burst (p =0.676 and p=0.904, respectively), but the chemotactic activity was increased (p=0.049) at T1.

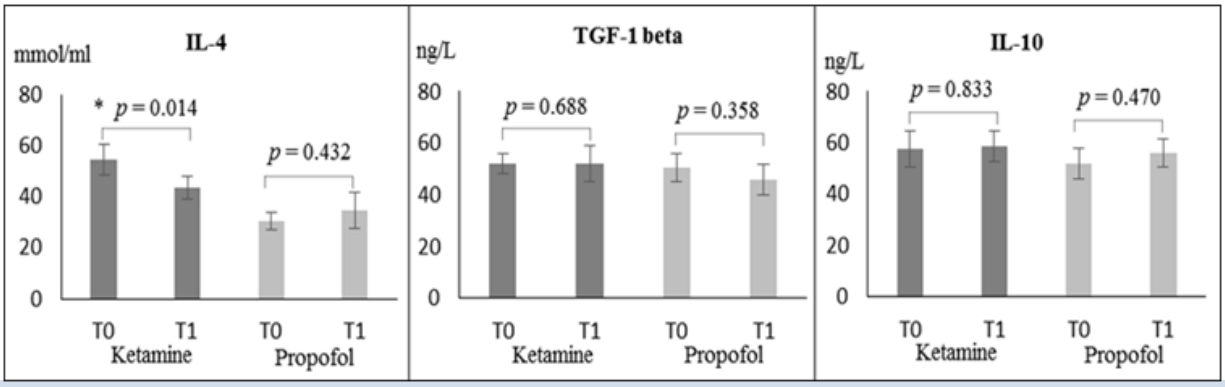

Figure 3. Mean values of interleukin-4 (IL-4), transforming growth factor-1 beta (TGF-1 beta) and interleukin-10 (IL-10) of dogs in ketamine and propofol groups; before sedation (T0) and 30 minutes after anesthesia induction (T1). The error bars show the standard error of mean (SEM). * Indicates that the difference between T0 and T1 is significant. n = 12 in ketamine and propofol groups

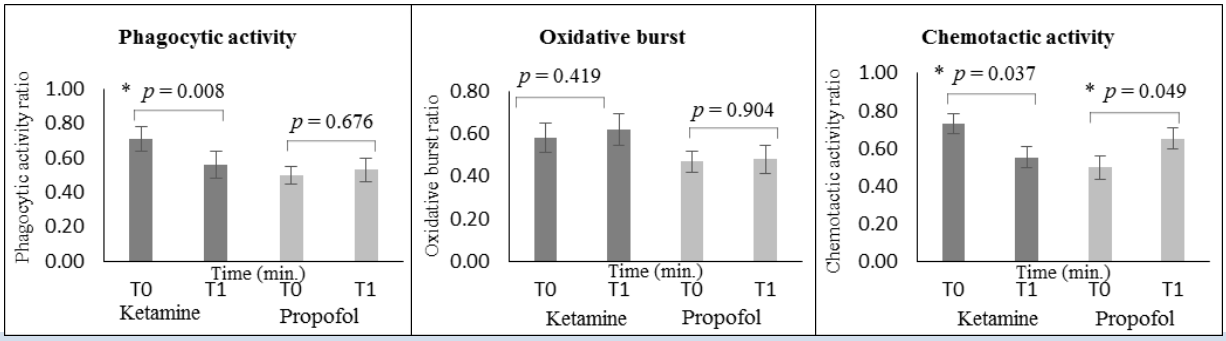

Figure 4. Mean values of phagocytic activity, oxidative burst, and chemotaxic activity of dogs in ketamine and propofol groups; before sedation (T0) and 30 minutes after anesthesia induction (T1). The error bars show the standard error of mean (SEM). * Indicates that the difference between T0 and T1 is significant. n = 12 in ketamine and propofol groups

DISCUSSION

The majority of anesthetics suppress the cardiorespiratory system by causing oxidative stress (Cruz et al., 2017). In this study, it was observed that anesthetics did not significantly affect HR but decreased RR (Table 2). Even though no apnoea occurred due to the initiation of anesthesia either with ketamine or propofol, it was considered that RR decreased with the effect of ongoing anesthesia. It is clear that respiratory suppression and a decrease in SpO_2 following administration of xylazine in premedication (Guzel et al., 2018). Therefore, changes in RR and SpO_2 following xylazine were not taken into consideration in this study. In the ketamine group, RR decreased but SpO_2 increased following 30 minutes after ketamine administration. This increase was that due to the dogs being ventilated with 100% oxygen after intubation. Despite the quantitative increase in SpO_2 with propofol at T1, this increasewas not found to be statistically significant, which is compatible with the references (McDonelland Kerr, 2007) indicating that propofol suppresses respiration as much as barbiturates whereas ketamine does not exert the same effect. In the study, $ETCO_2$value was found to be remained within the acceptable levels (McDonelland Kerr, 2007) in both groups. Hypothermia was not observed since the parameters were evaluated for a short duration (Guzel et al., 2018).

The most important defensive tasks are held by antioxidant enzymes such as SOD, CAT, and GSH-Px against the free radical injury in the body (Szczubialet al., 2015). Lupp et al. (1998) stated that ketamine suppresses the oxidative process by reducing lipid peroxidation, while Reinkeet al. (1998) reported that ketamine increased free radicals in serum. Kamiloglu et al. (2009) indicated that ketamine-xylazine anesthesia suppressed the free radical production and thus supported the antioxidant mechanisms. In this study, it was determined that MDA levels, which are the determinants of oxidative stress, decreased in the ketamine group, while SOD, CAT and GSH-Px levels did not change. This result showed that ketamine anesthesia reduced free radical formation and did not trigger oxidative stress. It was indicated that propofol increased GSH-Px level versus a decrease in MDA level (Akin et al., 2015).On the contrary, Tomsıč et al. (2018) stated that anesthesia with either propofol or sevoflurane did not affect the level of MDA, SOD and GSH-Px activities. In the study, propofol did not alter the levels of oxidative stress parameters such as SOD, CAT, GSH-Px, and MDA, which is compatible with the results of Tomsıč et al. (2018). It was evaluated that MDA levels in dogs receiving ketamine were found to be lower than those given propofol, and that ketamine anesthesia was more protective against oxidative stress.

Our data on cytokines shows that ketamine does not affect other proinflammatory cytokines studied except IL-6 (Figure 2). Previous studies have shown that IL-6 production, which is increased by using various stimuli (Yamaguchi et al., 2017) or as a result of surgical intervention (Welters et al., 2011), is suppressed by ketamine. It is possible to observe a similar effect in propofol. Thus, it is reported that propofol inhibits increased IL-6 biosynthesis as a result of lipopolysaccharide stimulation (Chen et al., 2005) or due to surgical intervention (Sayed et al., 2015). In this study, both ketamine and propofol evidentially decreased the IL-6 levels irrespective of either any cellular stimulation or the existence of surgical intervention (Figure 2). Normally, IL-6 is a pleiotropic cytokine that affects hepatocytes, megakaryocytes, neutrophils, T and B cells as well as synovial and dermal fibroblasts (Tanaka et al., 2014), which occurs as a result of the long-term exposure to IL-6 stimulation. In this study, it was considered more pertinent to assess the acute effects of IL-6 since it is a one-time application. In the early phases, IL-6 initiates the inflammatory reactions by increasing the expression of acute-phase proteins such as fibrinogen, c- reactive protein, and serum amyloid A or the adhesion molecules (ICAM-1, VCAM-1) (Barnes et al., 2011). Moreover, IL-6 causes apoptosis of neutrophils (McLoughlin et al., 2003). The suppression of IL-6 production by the anesthetics used in the study was found significant in terms of inhibition of possible inflammatory reactions and neutrophil apoptosis.

In this study, IFN- γ levels were significantly decreased in the propofol group unlike the ketamine group (Figure 2). It is highly unlikely to pronounce the precise cause of the relevant decrease in IFN- γ by propofol merely based on the available data. Nevertheless, T cells and NK cells were shown to be the major sources of IFN-γ (Ye et al., 1995). Supportively, it was reported that propofol inhibited T cell proliferation and activity by the aid of lymphocyte function-associated antigen-1 (LFA-1) (Yukiet al., 2011) and hence it was assumed that the decrease in IFN-γ might have been associated with the inhibition of T cells. However, it should be noted that the decrease in serum IFN-γ level might have been linked with the receptor status of IFN-γ since it was previously reported that propofol increased the expression of IFN-γ receptors on LPS stimulated whole blood cultures (Brand et al., 2001). An increase in IFN- γ receptors leads to the rapid elimination of IFN-γ from the circulation (Farrarand Schreiber, 1993). The decrease in serum IFN-γ after the induction of propofol occurred due to the depletion in secretion may be considered the downside of the protocol because IFN-γ undertakes critically important tasks such as the activation of macrophages, antigen presentation, upregulation of the non-specific immune response, and lymphocyte-endothelial cell interactions in the acute phases (Billiau, 1996). In the study, it was shown that propofol did not affect anti-inflammatory cytokines in dogs while ketamine decreased merely IL-4 levels (Figure 3). IL-4 is secreted from mast cells, Th2 cells, eosinophils, and basophils (Gadani et al., 2012). It was also reported that IL-4 adversely affected macrophages and dendritic cells (Hershey et al., 1997) and concordantly a decrease in IL-4 level with ketamine may be considered an affirmative effect because macrophages and dendritic cells are the most important antigen-presenting cells in the body. These cells are the link between the specific and the non-specific defence systems. One of the important properties of IL-4 is its capability to reduce Th1 cells (Hershey et al., 1997). Therefore, the decrease in IL-4 in the ketamine group was deduced to be a positive outcome since the de-

creased IL-4 secretion with ketamine did not exert a negative impact on Th1 cells in dogs, which was supported by the fact that ketamine did not decrease the levels of IFN-γ that is primarily produced by Th1 cells (Yang et al., 1999) and hence the IFN-γ levels were decreased in the propofol group unlike ketamine (Figure 2).

In vitro studies in humans (Helleret al., 1998) and dogs (Son et al., 2009) showedthat ketamine inhibited phagocytic and chemotactic activities of neutrophils. The inhibitory effect of ketamine on neutrophil functions has also been shown in vivo in the presented study, which raises the question of why ketamine reduces phagocytic and chemotactic activity. It is well known that mitochondrial ATP is required as the source of energy to maintain phagocytic and chemotactic activities (Son et al., 2009) and hence ketamine reduced the chemotactic activity by reducing mitochondrial membrane potential and thus ATP synthesis in previous in vitro studies (Chang et al., 2005). Therefore, it was considered that the reduction in phagocytic and chemotactic activities in the dogs that received ketamine in the study might have resulted from the suppression of the mitochondrial ATP production. Moreover, ketamine is known to inhibit pseudopod formation and elastase secretion in activated neutrophils (Craciun et al., 2013). Since pseudopod formation and elastase secretion play an important role in phagocytosis, the relevant concept was assumed to be one of the causes of the reduction in phagocytic activity of neutrophils in the study. Unlike ketamine, no reduction in neutrophil functions was detected in the dogs that received propofol. On the contrary, the chemotactic activity was increased (Figure 4). In vitro studies in humans demonstrated that propofol inhibited chemotactic activity and the other neutrophil functions through formyl peptide receptor-1 (FPR-1) (Yang et al., 2013). It was indicated that the relevant receptors are absent or are expressed in quite low quantities in dogs and some other animals (Linnekin et al., 1990). Therefore, it is little wonder that propofol did not inhibit chemotactic activity and vice versa in the dog, which was considered to be associated with a species-related diversity. However, there is no sufficient data available to precisely elucidate the underlying mechanisms.

CONCLUSIONS

In this clinical study, the anesthetic agents that were used had no effect on the cardiorespiratory system other than what was predicted.Thirty minutes after induction of anesthesia, ketamine prevented oxidative stress whereas propofol had no impact on the antioxidant defense system. The inhibition of IL-4 production by ketamine was considered to be a favorable outcome in regard to the defense system; however, the decreases in IL-6 levels, as well as the reduction in phagocytic and chemotactic capabilities of neutrophils were the adverse effects of ketamine. At the same time the suppression of IL-6 and IFN-γ production was an unfavorable effect of propofol on the defense system while the increase in the chemotactic activity of neutrophils was considered a positive aspect. In conclusion, it can be deduced that overall data obtained from the study should be noted while preparing anesthesia protocols particularly in terms of predicting possible postoperative reactions in the defense system. More research is needed in regards of the duration of the aforementioned results.

CONFLICT OF INTEREST STATEMENT

None of the authors has any other financial or personal relationships that could inappropriately influence or bias the content of the paper.

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