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Physiological changes, pain stress, oxidative stress, and total antioxidant capacity before, during, and after castration in male dogs

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ABSTRACT

The effect of duration on physiological change, pain stress, oxidative stress, and total antioxidant capacity before, during, and after castration of dogs was examined. A completely randomized design was used. Seven male dogs were castrated. Pain scores, physiological changes, and biochemical markers were investigated before, during, and for 14 days after castration of male dogs. Data were analyzed by using one-way analysis of variance. Results revealed that pain score, after 1 to 2 hours of recovery, was higher than that on days 3, 7, 10, and 14 of the experimental period ($P < 0.05$). Percentage of neutrophils, after dog's recovery and on day 3, was higher than that on day 14 of the experimental period ($P < 0.05$). Percentage of lymphocytes after dog's recovery was lower than that on day 3, before castration, and on days 7, 10, and 14 of the experimental period ($P < 0.05$). Neutrophil/lymphocyte ratio after dog's recovery was higher than that on day 3, before castration, and on days 7, 10, and 14 of the experimental period ($P < 0.05$). Heart rate and respiratory rate after dog's recovery were higher than that before castration on days 3, 7, 10, and 14 of the experimental period ($P < 0.05$). Total antioxidant capacity, before castration, after dogs recovery, and on day 3 was lower than that on day 14 of the experimental period ($P < 0.05$). This study indicated that the male dogs were under highest pain after recovery. At that time, they were under stress, but not under oxidative stress because their antioxidant system remained highly effective.

Introduction

The latest research indicates the first domestication of dogs occurred at least 40,000 years ago (Lallensack, 2017). In modern times, the domestic dog, *Canis lupus familiaris*, can be found living with and around humans throughout the globe (Shannon et al., 2015). Dog overpopulation and stray dog menace continues to be a significant problem worldwide. Castration is the most common operative procedure routinely performed on food and companion animals. To date, worldwide, castration is almost the sole method for control of pet overpopulation. Surgical removal of the testes is the method of choice for castration in dogs (Okwee-Acai et al., 2012). This method can prevent diseases of the reproductive system, such as benign prostatic hyperplasia, and modify undesirable behavior, such as urine marking and intermale aggression. However, castration has disadvantages and has had postoperative complications such as hemorrhage, wound dehiscence, infections, and scrotal swellings (Abd El- Wahed et al., 2014). Stress is a physiological response of animals that are up for adaptation after having been stimulated by stressors to maintain homeostasis in their body. Generally, animals responded to stressors in 4 aspects, namely, behavior, autonomic nervous system, neuroendocrine system, and immune system (Morberg and Mench, 2000). Surgical procedures represent a major source of stress for animals (Nenadović et al., 2017). Signals originating from stressor factors are transmitted to the hypothalamus in the brain, activating the hypothalamic-pituitary-adrenal and sympathoadrenal axes, which lead to release of glucocorticoids and catecholamines, respectively, that, through the induction of proinflammatory cytokines by macrophages and lymphocytes, promote the production of acute-phase proteins in hepatocytes and in the circulation in stressed animals. Moreover, it is known that a stressful condition leads to the imbalance between oxidants and antioxidants in favor of oxidants at the cellular or individual level inducing to oxidative stress that

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causes cellular damage, which makes the organism sensitive to serious degenerative diseases (Casella et al., 2013; Fazio et al., 2015; Passantino et al., 2014; Piccione et al., 2012). Hellyer et al. (2007) explained that all types of tissue injury can be generators of pain. The pain response involves two components: (1) the sensory component is nociception, which is the neural processing of noxious stimuli, and (2) the affective component is pain perception, which is the unpleasant sensory and emotional experience associated with either actual or potential tissue damage. Pain is the endpoint of nociceptive input and can only occur in a conscious animal; however, there is also involvement of autonomic pathways and deeper centers of the brain involved with emotion and memory. Reactive oxygen species (ROS) are produced by living organisms as a result of normal cellular metabolism. At low to moderate concentrations, they function in physiological cell processes (Birben et al., 2012). However, if ROS or free radicals are generated excessively or at abnormal sites, the balance between formation and removal is lost, resulting in oxidative stress (Yoshikawa and Naito, 2002). Higher production of ROS in the body or oxidative stress may change DNA structure, resulting in modification of proteins and lipids (Birben et al., 2012). Increasing evidence indicates that oxidative stress significantly impairs the function of organs and plays a major role in the etiology and pathogenesis of several diseases in humans and animals (Katerina et al., 2016). On the other hand, an antioxidant is a molecule stable enough to donate an electron to a rampaging free radical and neutralize it, thus reducing its capacity to damage. Moreover, antioxidants delay or inhibit cellular damage mainly through their free-radical scavenging property. These antioxidants can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged (Lobo et al., 2010). Although knowledge about pain stress, oxidative stress, and antioxidant status during, before and after castration in male dogs is limited, we have a hypothesis that castration may alter normal physiological function, some biochemical markers, and antioxidant status in male dog's body. Moreover, these parameters may change, before, during, and after operation. Therefore, the objective of the present study was to study physiological response, pain stress, oxidative stress, and total antioxidant capacity of male dogs during, before, and after castration. Results of this study will have important implications for dog during postcastration care and treatment.

Materials and methods

Experimental design

The experimental design of this study was completely randomized design with 6 treatments (times and number of dog treatments and replication) as follows: before castration, after dog's recovery, day 3, day 7, day 10, and day 14 of experimental period. During experimental period, dogs received feed and water *ad libitum*.

Methods

Animals

The study was conducted on 7 clinically healthy mixed-breed domestic male dogs (aged, during 1-3 years), with a mean ± standard deviation (SD) weight of 14 ± 5 kg. Animals were evaluated according to their history and absence of any previous illness. General clinical examinations, that is, physical examination, respiratory rate, heart rate, pulse rate, were performed and recorded. Blood samples of the experimental groups were taken from a cephalic vein for hematological analysis. All animals were administered conventional vaccination and deworming protocols and a fasting state of 6 hours before castration.

Sedation and anesthesia

Male dogs were sedated with a combination of xylazine 1.1 mg kg^{-1} (SC), tramadol hydrochloride 4 mg kg^{-1} (IM), 0.9% NSS 50 mL kg^{-1} (IV) with cefazolin sodium 22 mg kg^{-1} through an IV catheter, and then given thiopental sodium 10-25 mg kg^{-1} (IV) for 15 minutes until intubation. Dogs were maintained by administering thiopental sodium throughout surgical period.

Surgical procedure

Castration was performed according to the technique described by Tobias (2010).

Determination of pain score, physiological parameters, and laboratory analysis

Pain scores. Pain score assessment was performed by using the Glasgow Composite Measure Pain Scale. The form used was composed of a structured questionnaire completed by an observer following standard protocol which includes assessment of spontaneous and evoked behaviors, interactions with the animal, and clinical observations (Reid et al., 2007). This assessment was performed after dog's recovery, and on days 3, 7, 10, and 14 of the experimental period.

Physiological parameters. Rectal temperature, respiratory rate, and heart rate were recorded before castration, after the dog's recovery, and on days 3, 7, 10, and 14 of experimental period.

Hematological and biochemical parameters. Blood samples were collected before castration, after the dog's recovery, and on days 3, 7, 10, and 14 of the experimental period. The samples were taken from the saphenous vein using a butterfly needle into vacuum ethylenediaminetetraacetic acid and heparin tube for hematological and biochemical analysis, respectively. The blood in the heparin tubes was centrifuged at 2500 rpm (700× *g*) for 5 min, and the obtained heparinized plasma was frozen in cryotubes and stored at −20°C before biochemical analysis.

Blood cell differential count and calculation. Blood samples with added ethylenediaminetetraacetic acid as an anticoagulant were placed immediately on ice and transferred to the laboratory. Blood films were prepared, fixed with methanol, and stained with Giemsa-Wright solution, and then used for a white blood cell differential count. The neutrophil/lymphocyte ratio was calculated.

Plasma malondialdehyde. Malondialdehyde in plasma was investigated by using the following procedure: 0.01 mL of sample was assayed by the addition of 3 mL (0.05 mol/L) of HCl and 1 mL (0.67%) of thiobarbituric acid. Cocktails were heated for 30 mins at 100°C, cooled with running tap water, and then 4 mL of n-butyl alcohol was added. The mixture was shaken in a vortex mixer and centrifuged at 3,000 rpm (1008× *g*) for 10 min. The absorbance at 532 nm was compared with that of 1,1,3,3 tetramethoxypropane standard.

Plasma total antioxidant power. Plasma total antioxidant power was evaluated using the ferric reducing ability of plasma (FRAP) assay. The procedure was as follows: 300 mmol/L of acetate buffer (pH 3.6), 10 mmol/L of 2,4,6-tri-pyridyl-s-triazine in 40 mmol/L of HCl; and 20 mmol/L of $FeCl_3 \cdot 6H_2O$ were prepared. Twenty milliliters of acetate buffer, 2.5 mL of 2,4,6-tri-pyridyl-s-triazine, and 2.5 mL of $FeCl_3 \cdot 6H_2O$ yielded the working FRAP reagent, and then 10 μL of plasma, 10 μL of deionized distilled water, and working FRAP reagent were mixed. After exactly 6 minutes at room temperature, absorbance at 593 nm was read against reagent blank. Fe (II) at 100-1,000 μmol/L was used as standard.

Plasma catalase activity. Catalase activity was determined by spectrophotometric measurement of decreasing H_2O_2 quantity. Reagents were prepared as follows: 0.05 M potassium phosphate, pH 7.0, and 0.059 M hydrogen peroxide (30%) in 0.05 M potassium phosphate, pH 7.0. The spectrophotometer was adjusted to 240 nm at room temperature. A pipette was used to transfer 1.9 mL of reagent grade water and 1.0 mL of 0.059 M hydrogen peroxide to cuvettes. These were incubated in the spectrophotometer for 4-5 min to achieve temperature equilibration. After equilibration, 0.1 mL of sample was added and a decrease in absorbance at 240 nm for 2-3 min was recorded. Catalase enzyme was used as standard.

Statistical analysis

Data were analyzed by using one-way analysis of variance. Means were separated by Duncan's multiple range tests. All results were expressed as the mean ± standard deviation (SD). The level of significance was determined at $P < 0.05$.

Results

Physiological changes, stress and oxidative stress parameters, and total antioxidant capacity were investigated from 7 male dogs before castration, after the dog's recovery, and on days 3, 7, 10, and 14 of experimental period. The investigation yielded the following information: the pain score after the dog's recovery was significantly higher than that on days 3, 7, 10, and 14 of experimental period ($P < 0.05$). The percentage of neutrophils after the dog's recovery and on day 3 was significantly higher than that on day 14 of the experimental period ($P < 0.05$). After recovery, the percentage of lymphocytes was significantly lower than that on day 3, before castration and on days 7, 10, and 14 of the experimental period ($P < 0.05$). The neutrophil/lymphocytes ratio after recovery was significantly higher than that on day 3, before castration, and on days 7, 10, and 14 of experimental period ($P < 0.05$). Heart and respiratory rates after recovery were significantly higher than those before castration, on days 3, 7, 10, and 14 of the experimental period ($P < 0.05$). Total antioxidant capacity before castration, after dog's recovery on day 3, was significantly lower than that on day 14 of the experimental period ($P < 0.05$). Body temperature, plasma malondialdehyde, and catalase activity during the experimental period were not significantly different ($P > 0.05$) (Table).

Discussion

Generally, acute pain involves both nociceptive and inflammatory components and can be caused by trauma or surgery (Epstein et al., 2015). In this study, the pain score, after the dog's recovery, was higher than that before castration, on days 3, 7, 10, and 14 of the experimental period. This phenomenon showed that male dogs were under the highest pain after recovery. In this case, we classified pain from castration as acute pain because male dogs were under this condition for a short period. Heart rate and respiratory rate of male dogs after recovery were higher than those before castration, on days 3, 7, 10, and 14 of experimental period. This result was in accordance with the study of Ullah (2016) and Srithunyarat et al. (2016). They explained that during an animal's response to a stressor, the sympathoadrenal-medullary axis is stimulated and causes physiological changes such as increased heart and respiratory rate.

The percentage of neutrophils after the dog's recovery and on day 3 was higher than that before castration and on day 14 of the experimental period. The percentage of lymphocytes after recovery was lower than that on day 3, before castration, on days 7, 10, and 14 of the experimental period. The increasing percentage of neutrophils and the decreasing percentage of lymphocytes after recovery caused an increase in the neutrophil/lymphocyte ratio on day 3 and as higher than before castration on days 7, 10, and 14 of the experimental period. This phenomenon occurred under the influence of the catecholamines and glucocorticoids released after dog's surgery and under pain stress. In response to catecholamines and glucocorticoids, neutrophils shift from the marginated to the circulating neutrophil pool, but the neutrophilia might also be enhanced by the release of neutrophils from the bone marrow storage pool, while decreasing emigration of neutrophils to the tissues (Radisavljević et al., 2017). The percentage of lymphocytes decreased after the dog's recovery and was lower than that on day 3, before castration and on days 7, 10, and 14 of the experimental period. This result indicated that pain stress caused a decreasing percentage of lymphocytes. The decreasing of lymphocyte in this study was in accordance with the report of Bergeron et al. (2002). They found that injection of corticosteroids or adrenocorticotropic hormone in dogs caused an increase in neutrophils and a reduction in lymphocytes, the changes beginning 2 to 4 hours after injection. This hormone injection simulated a stress response in the animals that caused the decrease of lymphocyte.

Table
Effect of duration on physiological changes, pain scores, and biochemical markers before, during, and after castration in male dogs

Parameters	Before castration	After dogs's recovery	Day 3 after castration	Day 7 after castration	Day 10 after castration	Day 14 after castration	SEM
BW (kg)	14.49 ± 7.90	14.48 ± 7.92	14.51 ± 7.81	14.79 ± 7.84	14.78 ± 7.68	14.94 ± 7.55	2.94
Clinical Sign							
Pain Score	-	4.33 ± 1.75^{a}	1.00 ± 1.41^{b}	0.14 ± 0.38^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.37
Physiology							
HR (bpm)	104.14 ± 19.84^{b}	147.60 ± 24.39^{a}	107.43 ± 26.50^{b}	105.00 ± 17.65^{b}	104.57 ± 13.55^{b}	106.57 ± 21.76^{b}	7.91
RR (BPM)	41.17 ± 12.33^{b}	61.60 ± 13.76^{a}	35.71 ± 15.35^{b}	39.17 ± 4.40^{b}	37.17 ± 4.02^{b}	41.83 ± 8.01^{b}	4.04
BT (°F)	101.71 ± 0.43	101.26 ± 1.02	101.66 ± 0.90	101.97 ± 0.08	101.51 ± 0.55	101.43 ± 0.73	0.26
Hematology							
Neutrophil (%)	59.67 ± 5.13^{bc}	72.20 ± 8.87^{a}	71.67 ± 7.82^{a}	66.00 ± 6.40^{ab}	67.80 ± 4.32^{ab}	53.20 ± 3.11^{c}	2.38
Lymphocyte (%)	24.67 ± 5.35^{c}	5.00 ± 1.41^{e}	12.14 ± 4.88^{d}	28.80 ± 5.17^{bc}	34.00 ± 3.32^{b}	43.50 ± 5.43^{a}	1.77
N/L	2.73 ± 0.45^{bc}	17.61 ± 8.43^{a}	7.41 ± 3.09^{b}	2.52 ± 0.54^{bc}	2.08 ± 0.30^{c}	1.19 ± 0.18^{c}	1.20
Biochemistry							
MDA (μmol)	33.50 ± 3.71	36.98 ± 3.17	35.35 ± 9.06	34.58 ± 8.29	29.73 ± 9.07	29.15 ± 8.17	2.74
FRAP (mmol)	0.92 ± 0.10^{bcd}	0.86 ± 0.08^{d}	0.89 ± 0.09^{cd}	1.04 ± 0.15^{ab}	1.02 ± 0.13^{abc}	1.08 ± 0.15^{a}	0.00
Catalase (units/mg protein)	184.43 ± 119.08	197.29 ± 48.26	183.00 ± 40.26	184.43 ± 70.34	291.57 ± 76.90	198.71 ± 125.55	33.39

BW = body weight; HR = heart rate; RR = respiratory rate; BT = body temperature; N/L ratio = neutrophil/lymphocyte ratio; MDA = malondialdehyde; FRAP = ferric reducing ability of plasma.
Within rows, means with no common superscript differ significantly ($P < 0.05$).

Malondialdehyde is the most frequently used biomarker of oxidative stress (Dahake et al., 2016; Khoubnasabjafari et al., 2015). This biomarker is the best indicator of lipid peroxidation, indicative of oxidative stress. The antioxidants are the substances that reduce oxidation of substrates and constitute the body's main protection against free-radical injury. Oxidative stress is caused by more and more free-radical formation because of decreased level of antioxidants in the target cells and tissues. The levels of free-radical molecules are ruled by various cellular defense mechanisms consisting of enzymes such as catalysis. Reducing the oxidative stress by supplementation of antioxidant could be an effective option to prevent oxidative stress (Jain et al., 2015). In this study, the plasma malondialdehyde of male dogs, before castration, after recovery, on days 3, 7, 10, and 14 of experimental period was not different and was in accordance with catalase enzyme activity that did not differ throughout the experimental period. These occurrences indicated that dogs were not under oxidative stress after castration. Total antioxidant capacity (FRAP) of male dogs after recovery and on days 3, 7, and 10 was lower than that on day 14 of experimental period. It is possible that postcastration antioxidants were used for neutralizing or removing free radicals. Total antioxidant capacity of the dogs after recovery, on days 3, 7, and 10 was lower than that on day 14 of experimental period. This phenomenon showed that male dogs could benefit from antioxidants after castration.

In conclusion, after the dogs' recovered from castration, they were under pain stress because the pain score, percentage of neutrophils, neutrophil/lymphocyte ratio, and heart rate and respiratory rate had increased, whereas the percentage of lymphocytes decreased. On the other hand, dogs were not under oxidative stress because their antioxidant system still functioned with high effectiveness. This phenomenon was in accordance with the reduction of total antioxidant capacity. It is possible that male dogs need antioxidants after castration.

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Ethical considerations

This study was approved by the Institution's Ethics Committee on Animal Experimentation of Mahasarakham University (license number: 0029/2560). All procedures were performed with the owner's consent.

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