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Stress response related to ultrasonographic examination in dogs

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Abstract: This study investigated whether abdominal ultrasonography, a frequently used examination method in clinics and hospitals, causes stress in dogs. For the study, 10 healthy dogs were used. Following general examination, intravenous catheters were placed in the *V. cephalica accecorius* of the dogs and blood specimens were collected to obtain serum and plasma samples (minute 0). After a 30-min break, ultrasonography was applied following shaving of the abdominal region. Serum and plasma samples were collected from the intravenous catheters at the 5th, 15th, and 20th minutes of the ultrasonographic examination during which the urinary bladder, left kidney, spleen, stomach, liver, right kidney, transverse colon, and ascending colon were examined. The total antioxidant status (TAS), total oxidant status (TOS), nitric oxide (NOx), and cortisol levels of serum and plasma samples were analyzed and differences were statistically determined with respect to time. No statistically significant time-dependent changes in the TAS and cortisol values were observed. However, there was a time-dependent change in the TOS and NOx values, but when comparing all the times, no changes were observed. The results showed that abdominal ultrasonographic examination, a routinely and frequently used method, does not induce any stress factors and therefore cannot lead to health problems.

Key words: Dog, ultrasonography, oxidative stress

1. Introduction

Animal welfare is a state of mental and physical health in which the animal is in harmony with its environment and has adapted without suffering. In line with social needs, the welfare of nonhuman animals is of special concern to veterinary practitioners [1,2]. Many manipulations used in veterinary medicine during the animal production stage or in an effort to protect animal health can negatively affect animal welfare [3]. Stress, in general, is defined as the reaction of an organism to harmful environmental conditions that can lead to undesired consequences such as discomfort, or even death [4]. It can also be defined as the nonspecific reactions of an organism to internal and external factors, or the mutual interaction between stress elements and defense reactions [5]. Stress has been reported to cause problems that can affect life and productivity via lipid peroxidation, protein denaturation, and DNA mutations in cells [6].

Ultrasonography (USG) is a reliable, rapid, and repeatable examination method characterized by the use of sound waves that does not require any special preparation or administration of tranquilizers. It allows for the detection, screening, image recording, transferring to paper, and biopsy of intraabdominal pathologies. The USG is essentially a soft tissue examination method which allows for a clear inspection of the parenchymal organs in the abdomen such as the diaphragm, stomach, small intestines, large intestines, liver, spleen, pancreas, and kidneys. Such procedures provide information about the size, shape, location, position, and connections of these organs. It is also used in the identification of certain defects that cannot be detected with palpation, such as some abdominal masses, effusions, and intestinal peristaltic activity [7,8].

It was hypothesized that abdominal ultrasonography may cause stress in dogs. Therefore, the study investigated whether abdominal USG, a routinely and frequently used method in clinics and hospitals, causes stress factors in patients, and aimed to determine the mean time at which the examination method caused the lowest level of stress.

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2. Materials and methods

The study was performed under the approval dated 20.06.2017 and numbered 49533702 / 129 with reference number 252 - 17 of the local ethics committee for animal experiments of Afyon Kocatepe University.

For this study, from different breeds, a total of ten dogs of 1–9 years old brought to the AKU Faculty of Veterinary Medicine's Animal Hospital for ovariohysterectomy or castration were evaluated. A general examination of the healthy dogs was performed, and body temperatures and pulse and respiratory rates of the animals were measured. Intravenous catheters (22 G) were placed in the *V. cephalica accecorius* of the dogs, and blood specimens were collected to obtain serum and plasma samples (minute 0). After a 30-min break, USG was applied following shaving of the abdominal region. Serum and plasma samples were collected from the intravenous catheters at the 5th, 15th, and 20th minutes of the USG examination, during which the urinary bladder, left kidney, spleen, stomach, liver, right kidney, transverse colon, and ascending colon were examined.

The blood samples were centrifuged to separate plasma and serum (3500 rpm, 10 min, at room temperature) and kept at –20 °C until analyzed. The total antioxidant status (TAS), total oxidant status (TOS), nitric oxide (NOx), and cortisol levels were evaluated in the serum and plasma samples and the differences were statistically determined with respect to time. An Esaote My Lab Five VET-brand Doppler USG device with a microconvex probe screening at 5.0/8.0-MHz multifrequencies was used for the abdominal ultrasonographic examinations.

The TAS and TOS levels were determined using the spectrophotometric method with commercial kits. The NOx is a compound with a very short half-life that converts into nitrate and nitrite, which are its stable metabolites, with oxidation. Thus, the NOx level is usually measured by determining the levels of these metabolites. Therefore, the NOx amounts in the plasma samples were determined by following the vanadium chloride(III)–Griess reaction method [9] and plasma cortisol concentrations were determined using a cortisol ELISA kit.

Statistical analyses were performed using the PASW Statistics 18 package program. The Kolmogorov–Smirnov test was used to determine the normal distribution of the data. Logarithmic transformation was applied to data that did not show a normal distribution pattern. In the data analyses, repeated measurements for each variable were performed using one-way variance analysis. In the groups in which differences were determined, Bonferroni-adjusted pairwise comparison tests were used to determine from which groups the differences originated. Moreover, trend analyses (linear, quadratic, and cubic) were used to determine the characteristics of the changes in the groups in which changes were observed. The data are provided as mean ± SEM in the Table. Significance levels were accepted to be $P < 0.05$ in all analyses, except in the Bonferroni-adjusted pairwise tests.

3. Results

Mean body temperatures and mean pulse and respiratory rates were in physiological values in all dogs before the USG examination.

No statistically significant time-dependent changes in the TAS values were observed ($P > 0.05$). However, there was a time-dependent change in the TOS value ($P < 0.05$). There was a statistically significant increase at minute 5 compared with minute 0 ($P < 0.05$). The levels decreased at minutes 15 and 20 to the level at minute 0. The trend analysis showed that there was a quadratic change in the TOS value at minute 5 ($P = 0.039$). Furthermore, the highest TOS value was determined at minute 5 with a mean and standard deviation of 5.133 ± 0.362, while the lowest TOS value was determined at minute 0 with a mean and standard deviation of 3.240 ± 0.419 (Table; Figures 1 and 2).

There was also a time-dependent change in the NOx value ($P < 0.05$). There was a statistically significant, albeit slight, increase at minute 5 when compared with minute 0, and there was an increase at minute 15 when compared with minutes 0 and 5. The value at minute 20 decreased to the value at minute 5. The trend analysis showed that there was a linear change until minute 15 ($P = 0.0306$). Moreover,

Table. The TAS, TOS, NOx, and cortisol levels of the animals in the study group.

Parameters	Minute 0	Minute 5	Minute 15	Minute 20	P
TAS	0.320 ± 0.006	0.278 ± 0.015	0.293 ± 0.006	0.289 ± 0.007	0.094
TOS	3.240 ± 0.419^{a}	5.133±0.362^{b}	4.223 ± 0.371^{a}	4.195 ± 0.563^{a}	0.025
NOx (µmol/L)	21.134 ± 1.502^{a}	23.057 ± 1.685^{ab}	27.328 ± 1.577^{b}	26.628 ± 1.800^{ab}	0.016
Cortisol (nmol/L)	127.55 ± 13.60	142.18 ± 27.88	157.93 ± 35.43	149.64 ± 33.46	0.994

Different superscripted letters refer to significant differences between the values in the same rows ($P < 0.05$).

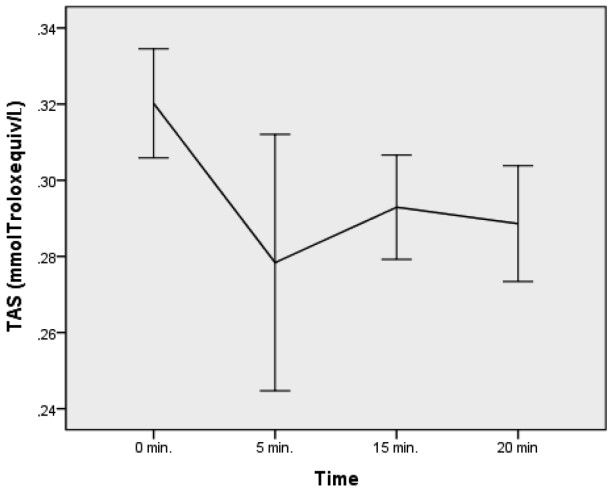

Figure 1. The TAS levels of the animals in the study group.

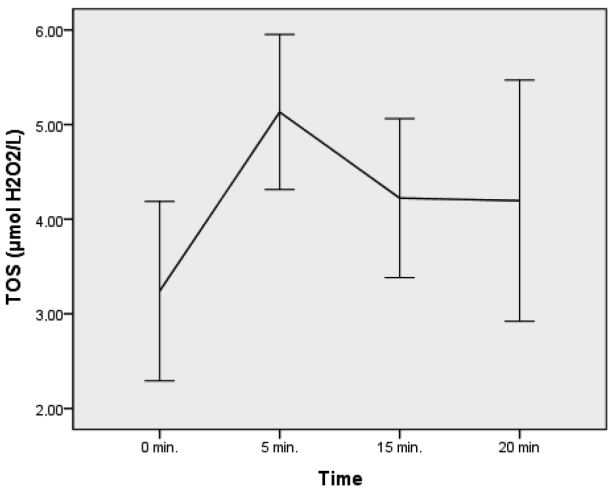

Figure 2. The TOS levels of the animals in the study group.

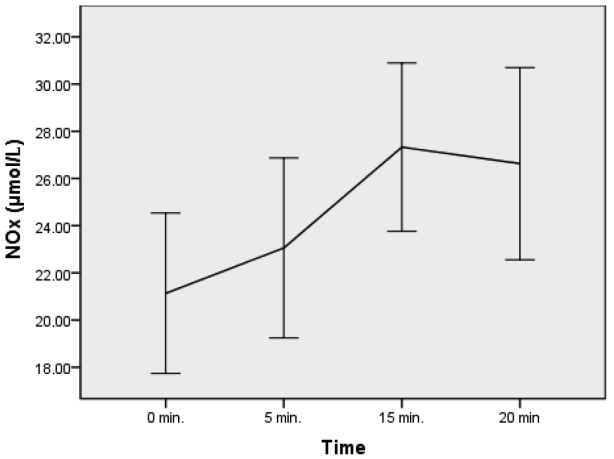

Figure 3. The NOx levels of the animals in the study group.

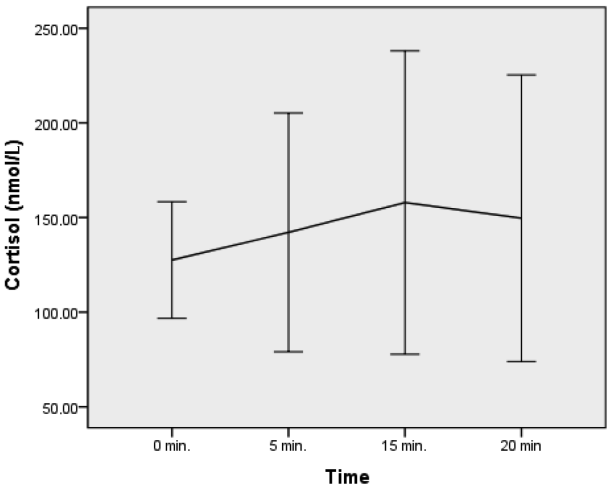

Figure 4. The cortisol levels of the animals in the study group.

the highest NOx level was determined at minute 15 with a mean and standard deviation of 27.328 ± 1.577, while the lowest NOx level was determined at minute 0 with a mean and standard deviation of 21.134 ± 1.502 (Table; Figure 3).

There were numerical time-dependent increases at other minutes compared with minute 0. The cortisol levels at minutes 5 and 15 were numerically increased, while the cortisol levels tended to decrease at minute 20 when compared with minutes 5 and 15; however, these time-dependent changes were not statistically significant ($P > 0.05$) (Table; Figure 4).

4. Discussion

In this study, the TAS and TOS measurements were used to determine the systemic effects of oxidative stress. The TAS is a suitable biochemical parameter for the determination of the overall antioxidant status of the serum and body fluids that stems from the intake and/or production of antioxidants and the levels of normal or increased reactive oxygen species production and consumption. The TAS and TOS measurements are believed to provide information as to the delicate balance between in vivo oxidants and antioxidants through the capacities and synergistic interactions of the known and unknown antioxidants [10,11]. In the oxidation events that occur in the body for different reasons, antioxidant defense systems are activated as part of a compensatory response. Constant and increasing production of the free radicals can weaken the antioxidant defense system [12]. On the other hand, when the oxidant/antioxidant balance is disrupted in favor of oxidants and oxidative stress occurs, a significant negative correlation

between the TAS and TOS values is observed [13]. It has been reported that the antioxidant system of an organism that is exposed to mild/moderate oxidative stress is activated at a more efficient level during the initial stage [14,15]. Consequently, the TAS levels decrease and, within this context, antioxidants with different structures are usually not observed during the initial stage and changes cannot be detected. Increases may even occur during this stage [16]. Not detecting the expected decreases in the USG-examined dogs can be explained in the light of these data. In addition, significant increases in the TOS levels of the healthy USG-examined dogs were observed at minute 5, which later decreased to levels that were not significantly different from the levels observed at minute 0. During the USG process, the formation of free radicals due to stress factors occurred briefly, and then they returned to normal. Again, compared with minute 0, no significant changes in the TAS levels occurred at minutes 5, 15, or 20 in response to the TOS levels. According to the results of the TAS and TOS levels, this also led to the conclusion that USG examination did not induce stress in the dogs.

Under conditions where animals are under physiological stress, a stress response that involves the sympathetic-adrenal-medulla axis (catecholamine release) and hypothalamic-pituitary-adrenal-cortex axis (releases corticosteroids) begins. This response causes changes that are viewed as stress indicators in hematological, biochemical, and clinical parameters [17]. Transport, dehorning, and rectal palpation procedures have been reported to indicate the occurrence of oxidative stress in ruminants [3,6,18]. Cortisol is a frequently used parameter in the evaluation of stress, animal welfare, and measurement of acute pain [19]. Furthermore, while humans can only hear sounds between 20 Hz and 20,000 Hz, dogs can hear ultrasonic sounds (40–60,000 Hz) as well. It has been reported that devices such as computer screens, surveillance cameras, and fluorescent lamps produce sounds that are not perceivable to human ears (>20,000 Hz) but can be heard by animals and thus can cause chronic stress [20]. In a similar manner, it has been noted that sudden loud sounds and ultrasonic sounds may cause stress in dogs [21] and increase serum cortisol levels in animals [22]. In this study, although a 20-min USG examination was performed, which was relatively long compared to routine applications, no significant differences in serum cortisol levels were observed, which led to the conclusion that routinely applied USG examinations of dogs do not cause any stress.

Nitric oxide is a small molecule that contains one unpaired electron and is synthesized through NOx synthase from L-arginine and oxygen due to the activation of various muscarinic and histamine receptors [23]. The role of NOx in organisms is controversial [24]. High NOx levels indicate tissue degradation [25] and can result in the emergence of certain potentially oxidative parameters such as hypotension, superoxide radicals, and inhibition of metabolic by products. NOx is a free radical that is defined as a biological signal molecule in processes such as neurotransmission, blood pressure regulation, defense mechanisms, smooth muscle relaxation, and immune regulation and plays important roles both in physiological and pathophysiological processes [23,26]. It is important in protecting the physiologic role of controlling excessive release and neutralization of excessive amounts without negatively affecting the basal release, and it can damage proteins, lipids, and DNA either directly or by reacting with superoxide, which leads to the formation of extremely reactive peroxide anions [24]. In this study, there were statistically significant time-dependent changes in the NOx values: there was a slight increase in the NOx levels at minute 5 when compared with minute 0 and there was a slight increase at minute 15 compared with minutes 5 and 0, after which the values returned to the levels at minute 5. The trend analysis showed that there was a linear change ($P = 0.0306$) in the NOx value until minute 15. The changes in the NOx values were simultaneous with the TOS levels but did not reach levels that can be considered an indicator of intense stress. Thus, it was concluded that USG examination can cause slight stress in patients during the first few minutes of the examination, but that stress did not cause health problems.

In conclusion, we are of the opinion that abdominal USG examination, a routinely and frequently used method in veterinary clinics and hospitals, does not induce any stress factors and therefore cannot lead to stress-related syndromes.

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