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Determination of Oxidative Stress and Antioxidant Activities in Dogs Infected with Canine Distemper Virus

Durmuş HATİPOĞLU[1,*] Irmak DİK[2] Erdem GÜLERSOY[3]

[1] *Department of Physiology, Faculty of Veterinary Medicine, University of Selcuk, 42075 Konya, Turkey*
[2] *Department of Virology, Faculty of Veterinary Medicine, University of Selcuk, 42075 Konya, Turkey*
[3] *Department of Internal Medicine, Faculty of Veterinary Medicine, University of Harran, 63200, Sanlıurfa, Turkey*

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ABSTRACT Supraphysiological reactive oxygen species (ROS) has been linked to a variety of diseases, including cancer, inflammation, and neurodegeneration. One of the diseases pathophysiologically associated with ROS accumulation is canine distemper (CD). The purpose of this study was to compare lipid peroxidation and antioxidant biomarkers in dogs infected with canine distemper virus (CDV) to healthy dogs. The animals in this study consisted of a total of 20 dogs, 10 CDV-positive dogs (Trial group) aged 0–12 months of different breeds and sexes, and 10 healthy dogs (Control group) aged 0–12 months of different breeds and sexes. Thiobarbituric acid reactive substances (TBARS), glutathione (GSH) and superoxide dismutase (SOD) were evaluated with commercially available enzyme-linked immunosorbent assay kits using serum samples. While the activities of GSH and SOD were statistically lower in CDV positive animals compared to healthy animals ($p<0.05$), the level of TBARS was significantly higher ($p<0.05$). In conclusion, current study findings that CDV increases lipid peroxidation activity in serum while decreasing antioxidant enzyme levels may be useful for future research.

Keywords: *Antioxidants, Canine distemper virus, Lipid peroxidation.*

ÖZ

Canine Distemper Virüs Enfeksiyonu Bulunan Köpeklerde Oksidatif Stres ve Antioksidan Aktivitelerin Belirlenmesi

Suprafizyolojik reaktif oksijen türleri (ROS), kanser, inflamasyon ve nörodejenerasyon dahil olmak üzere çeşitli hastalıklarla ilişkilidir. Patofizyolojik olarak ROS birikimi ile ilişkili diğer hastalıklardan biri de köpek distemperi'dir (CD). Bu çalışmanın amacı, canine distemper virüsü (CDV) ile enfekte köpeklerde lipid peroksidasyonu ve antioksidan biyobelirteçleri sağlıklı köpeklerle karşılaştırmaktı. Bu çalışmadaki hayvanlar, 0-12 aylık farklı ırk ve cinsiyetlerden 10 CDV pozitif köpek (Deneme grubu) ve 0-12 aylık farklı ırk ve cinsiyetlerden 10 sağlıklı köpek (Kontrol grubu) olmak üzere toplam 20 köpekten oluşmaktadır. Tiyobarbitürik asit reaktif maddeler (TBARS), glutatyon (GSH) ve süperoksit dismutaz (SOD) seviyeleri serumdan ticari olarak temin edilebilen enzim bağlantılı immünosorbent tahlil kitleri ile değerlendirildi. GSH ve SOD aktiviteleri, CDV pozitif hayvanlarda sağlıklı hayvanlara kıyasla istatistiksel olarak daha düşük iken ($p<0.05$), TBARS seviyesi önemli ölçüde daha yüksekti ($p<0.05$). Sonuç olarak, CDV'nin serumdaki lipid peroksidasyon aktivitesini artırırken antioksidan enzim düzeylerini azalttığına dair mevcut çalışma bulguları gelecekteki araştırmalar için faydalı olabilir.

Anahtar Kelimeler: *Antioksidanlar, Canine distemper virüsü, Lipid peroxidation.*

INTRODUCTION

Reactive oxygen species (ROS), which are formed due to the increase in oxidative stress in the cell, affect the double bonds of lipid and protein molecules as well as the double bonds of DNA bases, breaking a hydrogen atom from these and causing chain oxidation reactions to start, thereby disrupting their structure (Bedard and Krause 2007). The most crucial product formed in the last step of lipid peroxidation is malondialdehyde (MDA). MDA is one of the end products of polyunsaturated fatty acids peroxidation in cells (Mariutti 2022). The increase in free radicals causes overproduction of MDA. The presence of oxidative damage can be detected by measuring MDA, which is released because of oxidative damage to macromolecules by the effects of free radicals in body fluids and tissues by biochemical methods (Yazıcı et al. 2021; Avci et al. 2014; Hatipoglu and Keskin 2022). MDA can be chemically analyzed as a component of thiobarbituric acid reactive substances (TBARS) to assess

✉ *Corresponding author: drhatip@selcuk.edu.tr

lipid peroxidation (Gutteridge and Halliwell 1990; Dik et al. 2019). Glutathione (GSH) is the most critical low molecular weight antioxidant synthesized in cells. It is found in all mammalian tissues, especially in the liver (Lu 2009). The decrease in the level of GSH in the cell is due to the deterioration of the oxidant/antioxidant balance due to the decline of the defense mechanism against ROS. As a result, peroxidative tissue damage occurs. (Halder et al. 2016). Superoxide dismutase (SOD) is an enzyme that catalyzes the dismutation of superoxide radicals (O^2-) to molecular oxygen (O^2) and hydrogen peroxide (H^2O^2) and provides an effective defence against oxidative stress (Wang et al. 2018). SOD is the only antioxidant enzyme that scavenges the superoxide anion by converting this free radical to oxygen and hydrogen peroxide, thus preventing peroxynitrite production and further damage (Radi 2018).

Although physiological (beneficial) ROS confer several advantages in living organisms as signaling molecules, supraphysiological (harmful) ROS can cause harmful effects leading to oxidative damage to lipids, proteins, carbohydrates, RNA, and DNA. (Stuart et al. 2018; Sies and Jones 2020). Supraphysiological ROS has been associated with various diseases, including cancer, inflammation, and neurodegenerative diseases (Wang et al. 2021). One of the diseases pathophysiologically associated with ROS accumulation is canine distemper (CD) (Vandevelde and Zurbriggen 2005; Mahajan et al. 2018). CD caused by canine distemper virus (CDV) is a viral infection that affects the digestive, respiratory and central nervous systems (Beineke et al. 2009). CD is a severe immunosuppressive and neurological illness characterized by widespread demyelination lesions in the central nervous system's grey and white matter (Beineke et al. 2009). It is stated that these lesions are induced by virus replication and severe oxidative stress (Vandevelde and Zurbriggen 2005; Karadeniz et al. 2008) associated increased plasma concentrations of oxidative stress biomarkers with supraphysiological ROS accumulation, leading to disruption of antioxidant systems during CD. (Karadeniz et al. 2008).

This study aimed to determine lipid peroxidation and antioxidant biomarkers in dogs infected with canine distemper virus and compare them with healthy dogs.

MATERIAL AND METHODS

The study protocol was approved by the Ethics Committee (Veterinary Faculty, Selcuk University, Konya, Turkey, Decision No: 2020-124).

The animal material of this study consisted of 20 dogs in total which were brought to Animal Hospital either for diagnosis and/or treatment and routine check-up and/or vaccination purposes. All were aged between 0 and 12 months, unvaccinated client-owned mixbreed dogs. Anamnestic data revealed that clinical findings including nasal and ocular discharge, wheezing, and neurological symptoms such as tremors, myoclonus and chewing gum fits that would suggest the presence of CDV infection were present for at least 7 days.

The inclusion criteria of dogs suspected of being infected with CDV were based on anamnestic data, clinical examinations and rapid diagnostic test application results. To confirm suspicion of CDV infection, both ocular and nasopharyngeal secretions were obtained with sterile wet swabs and CDV antigen (Ag) test (Asan Easy Test CDV Ag®, ASAN Pharm. Co., Ltd. Gyeonggi-do Korea, relative sensitivity: 97.96%, relative specificity: 97.50%) was performed according to the manufacturer's instructions. Also, in order to rule out any concurrent diseases, Canine Adenovirus 2, Canine Influenza virus, Canine Coronavirus Ag (Asan Easy Test CAV^2 / CIV / CCV Ag®, ASAN Pharm. Co., Ltd. Gyeonggi-do Korea, relative sensitivity: 93.10%, relative specificity: 97.50%) tests were performed on all the dogs. All test results were determined to be negative. Moreover, microscopic fecal examinations of all dogs were performed with an appropriate method (zinc sulfate centrifugal flotation method for *Isospora* spp., centrifugal flotation method with a solution of sodium nitrate ($NaNO^3$, SG=1.32) for *Toxocara canis* and other parasite eggs), and all were determined to be negative as well. As a result, dogs with positive nasal/ocular CDV Ag test results were included in the Trial Group (n=10). Dogs with normal clinical findings and negative CDV, CAV^2, CIV, CCV Ag test results were considered healthy and included in the Control Group (n=10).

TBARS (TBARS Assay Kit, Cat. No: E0132Ca, BT LAB, China), GSH (Cat No: EA0021Ge, BT LAB, China) and SOD (Item No: 706002, Cayman USA) were evaluated with commercially available enzyme-linked immunosorbent assay kits using serum samples extracted from blood samples obtained using a jugular venepuncture technique from dogs with CDV and healthy dogs. Measurements were performed on an enzyme-linked immunosorbent assay reader (MWGt Lambda Scan 200, Bio-Tek Instruments, Winooski, VT, USA) according to the manufacturer's instructions.

Statistical Analysis

Data analysis was evaluated using SPSS 25.00 (SPSS for Windows®) statistical software and one sample Kolmogorov-Smirnov test was applied to determine whether all data were parametric or non-parametric. Data were evaluated using analysis of variance and a t-test as the post hoc test. A P-value of $p<0.05$ was accepted as the limit of statistical significance.

RESULTS

While a statistical decrease was noted in the activities of antioxidant parameters measured in CDV+ animals compared to healthy animals ($p<0.05$), a significant increase was found in the level of lipid peroxidation. ($p<0.05$).

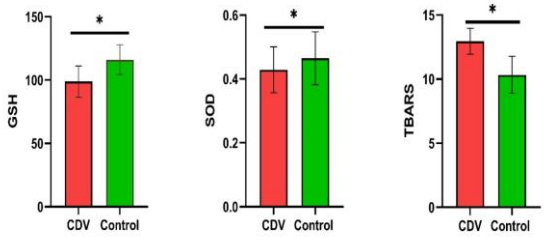

Figure 1: The changes in GSH, SOD and TBARS levels in CDV+ and control dogs. Values are expressed as mean ± SEM. The '*' symbol in the columns shows a statistical difference ($p<0.05$) when compared to the control group. (CDV+; Canine Distemper Virus Positive Group)

DISCUSSION AND CONCLUSION

Oxidative stress is defined as an imbalance in the body's generation of reactive oxygen species (ROS), which impairs the body's capacity to detoxify reactive

intermediates or repair damage to organs and cellular systems caused by ROS. (Finaud et al. 2006). However, since oxidative stress affects various systems, including redox signalling pathways, a more accurate description of oxidative stress is "disruption/dysregulation of signalling and redox control" (Jones 2006). Intracellular redox balance is closely related to antioxidant defence systems. When the redox balance is disturbed, reactive species can cause extensive damage to cell membrane lipids and proteins, DNA and cellular organelles (Burton and Jauniaux 2011). It has been determined that oxidative stress is formed, and antioxidant capacity is decreased due to various viral infections. (Paracha et al. 2013; Avci et al. 2014Ü; Değirmençay et al. 2021). The relationship between oxidative stress and antioxidant capacity in veterinary medicine in canine parvoviral enteritis (Panda et al. 2009), acute enteropathies (Candellone et al. 2022), canine monocytic ehrlichiosis (Pugliese et al. 2022), atopic dermatitis (Kapun et al. 2012) and malignant mammary neoplasms. This study aimed to reveal the relationship between CDV, oxidative stress, and antioxidant defence system mechanisms.

ROS accumulation is linked to the pathogenesis of canine distemper (Vandevelde and Zurbriggen 2005). Furthermore, Karadeniz et al. (2008) found that in dogs, the activation of oxidative stress indicators increased while the content of antioxidant molecules dropped. (Karadeniz et al. 2008). Current study results show that serum samples of dogs naturally infected with CDV have increased lipid peroxidation activity ($p<0.05$) and decreased antioxidant defence system enzyme levels compared to healthy dogs ($p<0.05$) (Figure 1). Previous studies indicate that CD infection causes oxidative stress and consequent lipid peroxidation, and by-products cause damage to various tissues (Değirmençay et al. 2021; Viscone et al. 2022). Viruses are thought to influence cellular redox balance by increasing the activity of oxidants like MDA and inhibiting the synthesis of antioxidant enzymes like SOD, CAT, and GSH (Camini et al. 2017). OS and lipid peroxidation products may interfere with viral reproduction by causing oxidative damage to host tissues and viral components (Schwarz 1996; Peterhans 1997; Beck 2000; Camini et al. 2017). Oxidative damage to infected and neighbouring cells may potentially limit viral propagation (Camini et al. 2017). However, the extent to which oxidative damage benefits the host by limiting viral replication is unknown for most viral infections (Valyi-Nagy and Dermody 2005). Moreover, it has been stated that at the start of the infection, ROS fights the disease, and the host views this as a protective mechanism that can lead the cell to the apoptotic pathway (Maher and Schubert 2000; Camini et al. 2017). It's claimed that as viral replication progresses, an imbalance in cellular redox homeostasis occurs, resulting in the formation of more ROS (Reshi et al. 2014). As a result, oxidative stress caused by viral infections is thought to modulate adhesion, metabolism, cell turnover, and death (Ha et al. 2010; Choi and Ou 2006; Camini et al. 2017). Based on the research thus far, it is assumed that oxidative stress is associated with various aspects of the pathogenesis of various viral etiological agents (Camini et al. 2017).

As a result, this study, which we found that CDV increases lipid peroxidation activity in serum and decreases antioxidant enzyme level, may be illuminating for further research. We think all aspects of the physiopathological mechanisms related to oxidative stress caused by CDV should be investigated. To better understand how the host responds to viral infection and CDV acts in the cell, a more detailed examination of signaling pathways, lipid peroxidation mechanisms, inflammatory responses, and antioxidant enzymes may be helpful to elucidate the therapeutic mechanisms that can use to fight and prevent CDV.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

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The study protocol was approved by the Ethics Committee (Veterinary Faculty, Selcuk University, Konya, Turkey, Decision No: 2020-124).

AUTHOR CONTRIBUTIONS

Idea / Concept: ID, EG
Supervision / Consultancy: ID, DH
Data Collection and / or Processing: EG
Analysis and / or Interpretation: DH, ID
Writing the Article: DH
Critical Review: ID, EG

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