Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
E-ISSN: 1309-2251

Kafkas Univ Vet Fak Derg
31 (4): 547-556, 2025
DOI: 10.9775/kvfd.2025.34251

Research Article

Oxidative Stress in Neurological Feline Infectious Peritonitis: Cerebrospinal Fluid 8-Hydroxy-2'-deoxyguanosine and Superoxide Dismutase Levels

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How to cite this article?

Baştan İ, İrdem Dİ, Sel T, Kaya Kartal Y, Ergin SH, Tunç AS: Oxidative stress in neurological feline infectious peritonitis: Cerebrospinal fluid 8-hydroxy-2'-deoxyguanosine and superoxide dismutase levels. *Kafkas Univ Vet Fak Derg,* 31 (4): 547-556, 2025.
DOI: 10.9775/kvfd.2025.34251

Article ID: KVFD-2025-34251
Received: 16.04.2025
Accepted: 30.07.2025
Published Online: 05.08.2025

Abstract

Oxidative stress plays a key role in the pathogenesis of neurological disorders and viral infections affecting the central nervous system. 8-Hydroxy-2'-deoxyguanosine (8-OHdG) is a marker of oxidative DNA damage, while superoxide dismutase (SOD) reflects antioxidant defense. This study aimed to evaluate SOD and 8-OHdG levels in the cerebrospinal fluid (CSF) of cats with neurological feline infectious peritonitis (FIP) to assess oxidative stress and antioxidant response. Twelve cats with neurological FIP and 10 age-matched control cats euthanized for non-neurological conditions were included. FIP diagnosis was confirmed by detecting feline coronavirus (FCoV) RNA in the CSF using real-time RT-PCR and by histopathological examination. CSF samples were analyzed for total protein, glucose, SOD, and 8-OHdG. Cats with FIP showed significantly higher CSF protein (740±230 mg/dL) than controls (17±7 mg/dL). The CSF/serum glucose ratio was lower in FIP cats (0.39±0.18) than in controls (0.66±0.06). 8-OHdG levels were elevated in FIP cats (6.88 ng/ml) compared to controls (1.09 ng/ml; $P<0.05$). SOD levels were reduced in FIP cats (0.034±0.026 U/mg protein) versus controls (0.312±0.136 U/mg protein; $P<0.001$). These findings highlight a pronounced oxidative stress condition in neurological FIP, characterized by elevated 8-OHdG levels and reduced SOD concentrations in the CSF. This concurrent pattern may not only serve as a valuable biomarker of disease activity but also represent a potential therapeutic target for antioxidant-based strategies in affected cats.

Keywords: 8-OHdG, Cat, Cerebrospinal fluid, Feline infectious peritonitis, Oxidative stress, Superoxide dismutase

Introduction

Feline infectious peritonitis (FIP) is a progressive disease caused by a mutant biotype of feline coronavirus (FCoV) in domestic cats. It presents with a wide spectrum of clinical manifestations, ranging from effusive to no effusive forms, with neurological involvement occurring in approximately one-third of affected cats. The neurological form of FIP is the most common infectious disease affecting the feline central nervous system (CNS) and is characterized by multifocal inflammatory lesions involving the brain and spinal cord. These lesions frequently manifest as meningitis, choroid plexitis, ependymitis, and periventriculitis, potentially leading to obstructive hydrocephalus or hydromyelia. Clinically, neurological FIP can present with seizures, compulsive behaviors, cognitive impairment, and urinary or fecal incontinence [1-3].

The CNS is particularly vulnerable to oxidative stress due to its high metabolic rate, lipid-rich composition, and limited antioxidant defenses. Reactive oxygen species (ROS), generated as byproducts of cellular metabolism and viral infections, contribute to neuronal injury and neurodegeneration. While enzymatic and non-enzymatic antioxidant systems protect against ROS-induced damage, these defenses may be insufficient to prevent oxidative injury in pathological conditions. Superoxide dismutase (SOD), a key endogenous antioxidant enzyme, plays a

critical role in mitigating oxidative stress by catalyzing the dismutation of superoxide radicals. Conversely, oxidative DNA damage is frequently assessed using 8-hydroxy-2'-deoxyguanosine (8-OHdG), a well-established biomarker of oxidative stress and DNA repair processes [4,5].

Oxidative stress and antioxidant imbalance have been extensively investigated in human and experimental CNS diseases, where they are known to contribute to neuronal damage and have been explored as potential therapeutic targets [4,5]. In feline medicine, however, the involvement of oxidative mechanisms in neurological conditions such as FIP has not been fully elucidated. In this study, CSF concentrations of 8-OHdG, a marker of oxidative DNA damage, and SOD, a key antioxidant enzyme, were evaluated in cats with neurological FIP. By assessing oxidative stress and antioxidant responses in this context, the study aims to contribute to the current understanding of the disease's pathophysiology and explore the potential relevance of these markers in clinical evaluation and future research.

Material and Methods

Ethical Statement

This study was carried out after the animal experiment was approved by Ankara University Local Ethics Committee of Animal Experiments (Decision number: 2024-19-156; Date: 11/12/2024). Written informed consent was obtained from the owners of each animal prior to their inclusion in the study.

Study Population and Sample Processing

This study included 12 cats diagnosed with the neurological form of FIP and 10 age-matched control cats without any CNS pathology. All cats included in the study were presented to the Ankara University Veterinary Faculty Hospital from October 2021 to October 2023 and euthanized because of poor prognosis. Euthanasia recommendations were independently made by two veterinarians who were not involved in the study, and informed consent was obtained from the owners. Cats showing neurological signs suggestive of FIP and a poor prognosis were included in the FIP group. Control cats had similarly poor prognoses but no neurological signs.

A complete clinical history was recorded for all cats, followed by thorough physical and neurological examinations. Cerebrospinal fluid samples were collected from the cerebellomedullary cistern, and full necropsies with histopathological examination were performed after euthanasia.

Blood samples were collected from all cats prior to euthanasia. After clotting, serum was separated by centrifugation and used for biochemical analysis. The analyses were performed immediately after sample collection. Serum concentrations of total protein, albumin, and glucose were measured using an automated biochemistry analyzer (Randox RX Monaco, Randox Laboratories) at the Diagnostic Laboratory of the Faculty of Veterinary Medicine, Ankara University. Standard colorimetric and enzymatic assay methods were used, following the manufacturer's protocols. For CSF collection, cats were anesthetized via intramuscular administration of medetomidine HCl (Domitor® 1 mg/mL; Orion Pharma, Espoo, Finland) and ketamine HCl (Ketasol® 100 mg/mL; Interhas, Ankara, Türkiye) to ensure adequate immobilization. The dorsal cervical region was shaved and aseptically prepared for cerebellomedullary cisternal puncture. CSF was collected using a 19G spinal needle, and a minimum of 2 mL was transferred into sterile Eppendorf tubes. Each CSF sample was divided into three aliquots. One of the two aliquots was immediately frozen at –80°C for subsequent biomarker analysis, while the second was stored as a backup sample under the same conditions. The third aliquot was kept under cold chain conditions (4°C) and promptly delivered to the laboratory for the measurement of total protein, glucose concentration, and real-time RT-PCR analysis. Euthanasia was performed using T61® (MSD; containing 200 mg embutramide, 50 mg mebezonium iodide, and 5 mg tetracaine hydrochloride per mL).

CSF total protein concentration was measured using the biuret method, based on the reaction of copper ions with peptide bonds in the protein molecule, forming a complex that produces a violet color [6]. Glucose concentration was measured using a UV test method based on the enzymatic trinder's method. Glucose in the sample is oxidised to yield gluconic acid and hydrogen peroxide in the presence of Glucose oxidase. The enzyme peroxidase catalyses the oxidative coupling of 4-aminoantipyrine with phenol to yield a coloured quinonemine complex, with absorbance proportional to the concentration of glucose in sample [7].

All cats were screened for feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) using a commercial antibody test kit (GenBody FeLV-FIV®, GenBody Inc., South Korea). In addition, Toxoplasma gondii serology was performed using a semi-quantitative immunoassay (ImmunoComb® Toxoplasma, Biogal, Israel).

Inclusion in the FIP group required the presence of neurological signs, a low serum albumin-to-globulin (A/G) ratio (<0.6), detection of FCoV RNA in the CSF via RT-PCR, and histopathological confirmation of FIP. Control cats showed no evidence of CNS involvement. Cats from the control group were excluded if CNS lesions were identified at necropsy. In both groups, cats that tested positive for feline leukemia virus, feline immunodeficiency virus, or *Toxoplasma gondii*, or those showing signs of

multisystemic disease, were excluded from the study. RT-PCR analysis confirmed the absence of FCoV RNA in the CSF of control cats. None of the cats received antioxidants, nonsteroidal anti-inflammatory drugs, or corticosteroids following the onset of clinical signs.

Histopathological Assessment

Following euthanasia, necropsies were performed on all cats in both the FIP and control groups. Brain and spinal cord tissues were carefully taken and fixed in 10% neutral-buffered formalin for at least 48 h. After fixation, samples were routinely processed, embedded in paraffin, and sectioned at 4 µm thickness. Histological sections were stained with hematoxylin and eosin (HE), evaluated under light microscopy (Leica DM 4000) and photographed (Leica DFC-280). Central nervous system tissues were examined for histopathological lesions consistent with FIP, including perivascular inflammation, pyogranulomatous areas, and meningoencephalitis/meningoencephalomyelitis. Tissues from the control group were also examined to confirm the absence of CNS pathology.

RNA Extraction

Viral RNA was extracted from the cell-free fraction of CSF samples using the QIAamp Viral RNA Mini Kit® (Qiagen, Hilden, Germany), following the manufacturer's recommended protocol. In brief, 140 µL of each CSF sample was mixed with lysis buffer under highly denaturing conditions to ensure the complete inactivation of RNases. The lysed mixture was then applied to silica spin columns, where viral RNA selectively bound to the membrane. After sequential washes to remove impurities, the RNA was eluted in 60 µL of RNase-free elution buffer and stored at −80°C until further analysis.

Real-Time RT-PCR

The presence of FCoV RNA in CSF was assessed using a one-step real-time reverse transcription PCR (RT-PCR) [8]. Reactions were set up using the QuantiTect Probe RT-PCR Kit® (Qiagen, Germany) in a final volume of 25 µL. Each reaction included 5 µL of extracted RNA, 12.5 µL of 2X Master Mix, 0.25 µL of reverse transcriptase mix, 2 µL of a primer–probe mix specific for the FCoV 3′ untranslated region (UTR), and 5.25 µL of RNase-free water.

Primers were used at a final concentration of 0.8 µM, and a TaqMan hydrolysis probe labeled with 5′-FAM and 3′-BHQ-1 was used at 0.3 µM. The thermal cycling protocol consisted of reverse transcription at 50°C for 30 min, followed by enzyme inactivation and initial denaturation at 95°C for 15 min. This was followed by 42 amplification cycles of denaturation at 95°C for 30 sec and combined annealing and extension at 60°C for 60 sec. Amplification and real-time fluorescence detection were performed using a Stratagene Mx3005P® (Thermo Scientific, USA) instrument. Samples were considered FCoV-positive when a specific amplification curve crossed the threshold level before cycle 42.

8-Hydroxy-2'-deoxyguanosine (8-OHdG) Analysis

This test was done according to the steps of the ELISA kit instructions. The kit principle uses the competitive ELISA method (Elabscience®, cat no: E-EL-0028). The intra and inter assay CV of the kit was <8% and <10%. The sensitivity of the test was 0.94 ng/mL and the detection range was between 1.56 and 100 ng/mL. The micro-ELISA plate provided in this kit has been precoated with 8-OHdG. During the reaction, 8-OHdG in the sample or standard competes with a fixed amount of 8-OHdG on the solid phase supporter for sites on the Biotinylated Detection Ab specific to 8-OHdG. Excess conjugate and unbound sample or standard are washed from the plate and Avidin conjugated to Horseradish Peroxidase (HRP) are added to each well and incubated. Then A TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of stop solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of 8-OHdG in the samples is then determined by comparing the OD of the samples to the standard curve.

Superoxide Dismutase (SOD) Analysis

Analysis was carried out according to the method developed by Sun et al.[9]. For superoxide dismutase activity, it involves the inhibition of nitro blue tetrazolium reduction by xanthine - xanthine oxidase used as a superoxide generator. The OD values were read at 560 nm and first the % of the inhibition was calculated with the formula given; % inhibition = (Blank OD. – Test OD)/Blank OD) x 100. A Unit of SOD has an inhibition activity for 50% so this calculation can give the Unit of SOD in 1 mL. By dividing Unit to protein, the result is given as U/mg protein.

Statistical Analysis

SPSS 21.00 packet programme was used for the statistical analysis. According to the Normality contribution (Shapiro wilk), independent samples T test (Me±SD) or Mann Whitney U test (Median (Q1-Q3)) was chosen and $P<0.05$ were accepted as important. The results were given as Me±SD and Median (Q1-Q3) in *Table 1*. The statistical test according to the normality contribution was Mann Whitney U test for CSF glucose/serum glucose, 8-OHdG and SOD measurements, while independent samples t-test was for age, CSF total protein and serum Alb/ serum glb measurements. In the result section Median (Q1-Q3) concentrations were used for 8-OHdG because of the large SD value.

Results

FCoV RNA was detected in all 12 CSF samples from FIP cats, while all 10 samples from the control group tested negative.

Of the 12 cats in the study group, 5 (41.7%) were female, and 7 (58.3%) were male. In the control group, 4 out of 10 cats (40%) were female, and 6 (60%) were male. There was no statistically significant difference in age between the study and control groups (P>0.05) *(Table 1)*. In the control group, four cats were euthanized due to atrial thromboembolism, while six were euthanized as a result of pneumothorax and pulmonary contusion secondary to high-rise syndrome.

Neurological examination findings of 12 cats diagnosed with neurological FIP included anisocoria, blindness, nystagmus, fascial paralysis, decreased gag reflex, paresis, paralysis, cross-extension reflex, ataxia, decerebellar rigidity, hyperesthesia, decreased to absent menace response, proprioceptive deficits, urinary incontinence, fecal incontinence *(Table 2)*.

Cats with FIP had higher CSF Total Protein (740±230 mg/dL) compared to control group (17±7 mg/dL). CSF glucose/serum glucose (mg/dL divided by mg/dL) ratio was significantly lower in cats with neurological FIP compared to the control group (0.39±0.18, 0.66±0.06, respectively). The median (Q1-Q3) concentration of 8-OHdG in CSF samples of cats with FIP (6.88 [2.13-48.20] ng/mL) was significantly higher than in control subjects (1.09 [0.33-3.56] ng/ml; P<0.05). Cerebrospinal fluid levels of SOD were significantly decreased in cats with FIP (0.034±0.026 U/mg) compared to controls (0.312±0.136, P<0.001) *(Table 2)*.

Histopathological examination of the brains of cats with FIP revealed hyperemia in vessels, perivascular mononuclear cell infiltration, edema, and necrosis in meninges *(Fig. 1-a)* and choroid plexus *(Fig. 1-b)*.

Internal hydrocephalus occurred in some cats. Gliosis and demyelination were noted, especially in the substantia alba.

Table 1. *Comparison of age, CSF biochemical parameters, oxidative stress markers, and serum albumin/globulin ratio between cats with feline infectious peritonitis (FIP) and healthy controls*

Parameters	Groups				P
	Cats with FIP		Control		
	Mean ± SD	Median (Q1-Q3)	Mean ± SD	Median (Q1-Q3)	
Age (Year)	2.35±0.57	2.25 (2.00-3.00)	2.24±0.48	2.45 (1.75-2.60)	>0.05
CSF Total Protein (mg/dL)	740±230	780 (510-920)	17±7	14 (11-22)	<0.001
CSF Glu/Serum Glu (mg/dL divided by mg/dL)	0.39±0.18	0.41 (0.25-0.45)	0.66±0.06	0.65 (0.60-0.72)	<0.001
8-OHdG (ng/mL)	24.38±34.32	6.88 (2.13-48.20)	5.73±13.26	1.09 (0.33-3.56)	<0.05
SOD (U/mg protein)	0.034±0.026	0.027 (0.017-0.041)	0.312±0.136	0.285 (0.238-0.350)	<0.001
Serum ALB/GLB	0.35±0.15	0.30 (0.23-0.50)	0.87±0.06	0.86 (0.82-0.90)	<0.001

P-values <0.05 were considered statistically significant

Table 2. *Neurologic examination findings of cats with FIP*

Neurologic Examination Findings	Number of Cats with Clinical Signs	Percentage of Cats with Clinical Signs
Anisocoria	4	33.3%
Blindness	2	16.6%
Nystagmus	5	25.0%
Fascial paralysis	2	16.6%
Decreased gag reflex	2	16.6%
Paresis	5	41.6%
Paralysis	1	8.3%
Cross-extension reflex	10	83.3%
Ataxia	5	41.6%
Decerebellar rigidity	3	25.0%
Hyperesthesia	5	25.0%
Decreased to absent menace response	4	33.3%
Proprioceptive deficits	10	83.3%
Urinary incontinence	8	66.6%
Fecal incontinence	8	66.6%

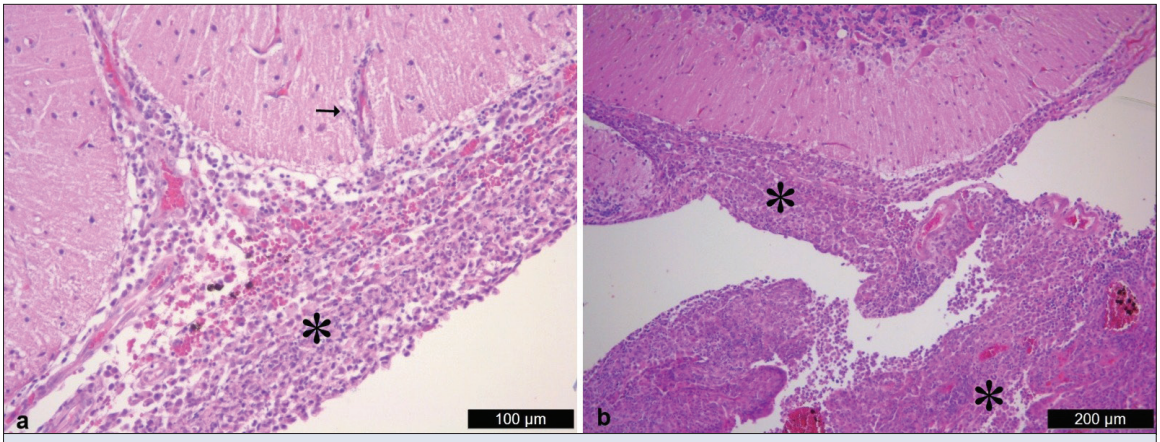

Fig 1. (a) Meningitis *(asterisk)* composed of mononuclear cells and perivascular cell infiltration *(black arrow)*, cerebellum, HE, **(b)** Inflammation characterized by mononuclear cells *(asterisk)*, choroid plexus, HE

Discussion

In this study, CSF total protein concentration was significantly elevated, whereas CSF glucose concentration were markedly reduced in cats with neurological FIP compared to the control group. The inflammatory response, including cytokine and nitric oxide metabolite production, likely disrupt mitochondrial function, increasing glucose consumption through anaerobic glycolysis and resulting in reduced CSF glucose levels and impaired brain function [10,11]. Cerebrospinal fluid proteins are derived from serum and local intra-cranial synthesis. Disruption of the blood-brain barrier increases permeability, allowing increased levels of serum proteins to access the CSF [12]. Inflammation, viral replication, and altered vascular permeability associated with neurological feline infectious peritonitis (FIP) contribute to elevated CSF protein and albumin levels [13-17]. These findings are consistent with previous reports, including those by Chrisman [13], Deisenhammer et al.[14], DiTerlizzi and Platt [15], Regeniter et al.[16] and at least Singh et al.[17] suggesting that severe inflammation and immune-mediated damage in neurological FIP contribute to elevated CSF protein levels and reduced glucose concentrations.

8-Hydroxy-2'-deoxyguanosine is recognized as the most prevalent oxidatively damaged product resulting from DNA exposure to free radicals, and it serves as a reliable biomarker for assessing the extent of oxidative DNA damage [18-20]. In the present study, histopathological examination of the CNS of cats with FIP revealed inflammatory lesions consistent with previous descriptions of neurological FIP [21-24]. These inflammatory changes, including perivascular infiltration of macrophages, lymphocytes, and neutrophils, are known to contribute to oxidative stress within the CNS microenvironment [25]. The inflammation-associated oxidative burden observed in our study is in agreement with the findings of Tecles et al.[26], who demonstrated the presence of oxidative stress in the serum of cats with FIP. In our study, significantly increased levels of 8-OHdG were detected in the cerebrospinal fluid of cats with neurological FIP. This observation may suggest the involvement of localized oxidative DNA damage within the central nervous system. These combined findings support the role of inflammation-induced oxidative damage as a contributing factor in the neuropathogenesis of feline infectious peritonitis. Oxidative stress of DNA contributes to early neuronal damage [27]. Increased levels of oxidative stress have been reported in the CSF of patients with various neurodegenerative disorders including Alzheimer's disease [28], Parkinson's disease [29], Amyotrophic Lateral Sclerosis (ALS) [30] and Multiple Sclerosis (MS) [31], as well as infectious diseases such as bacterial and aseptic meningitis [32], encephalitis associated with Influenza A [33] and Herpes Simplex Virus [34]. In the present study, we demonstrated that CSF-8-OHdG levels in cats with neurologic FIP were significantly higher than in controls, suggesting that DNA oxidative stress is induced by neurological FIP infection.

Recent studies have identified oxidative damage as a fundamental mechanism underlying central nervous system injury induced by viral infections such as herpes simplex virus type 1 in murine [35,36], human herpesvirus-6 (HHV-6) [37]. Oxidative injury is a significant component of acute encephalitis induced by herpes HSV-1 [38]. In neurological FIP, several cytokines are consistently elevated, indicating a strong inflammatory response, which contributes to oxidative stress within the CNS [23]. The increase in ROS may occur due to a mitochondrial dysfunction caused by penetration of the virus into the cell or by signaling exacerbated by the "cytokine storm" with release of IL-2, IL-6, IL-7, and TNF-α [39]. Similarly, immune cells such as macrophages and neutrophils play a potential pathological role by producing and secreting high pro-inflammatory cytokines and ROS levels [40]. Cells utilize antioxidant defense mechanisms, including

the activation of enzymes such as SOD to counteract ROS-induced damage. Notably, increased activities of both SOD and catalase in CSF have been observed in patients with chronic neurodegenerative diseases [41,42], bacterial meningitis [32] and malaria [43]. Under normal physiological conditions, a dynamic equilibrium is maintained between ROS production and the antioxidant enzyme system. When the balance between ROS production and antioxidant defense is disrupted, excessive ROS can induce oxidative modifications in lipids, proteins, and polysaccharides, ultimately resulting in DNA and RNA damage [44,45]. In the present study, CSF 8-OHdG levels were significantly elevated in cats with FIP compared to controls, whereas CSF SOD concentrations were markedly reduced. The regulation of ROS activity is maintained by a complex antioxidant system that modulates intracellular ROS levels. However, under prolonged oxidative stress, ROS concentrations surpass the scavenging capacity of the antioxidant defense system, leading to extensive cellular damage and necrosis [46]. The decreased CSF-SOD activity observed in FCoV-infected cats may reflect depletion resulting from excessive oxidative burden within the CSF. Specifically, the heightened oxidative stress and inflammation associated with FIP may have resulted in the substantial consumption of SOD as it attempted to counteract ROS-mediated damage in CNS. Alternatively, the observed reduction in SOD levels may not only be due to enzymatic depletion caused by excessive oxidative stress but also reflect coronavirus-mediated immuno-suppression or direct viral interference with antioxidant gene expression. Previous studies have shown that several viruses, including coronaviruses can suppress antioxidant defense by downregulating SOD transcription or disrupting the nuclear factor erythroid 2-related factor 2 pathway, a key regulator of cellular antioxidant responses [47-49].

Elevated levels of free radicals, in conjunction with diminished antioxidant defenses, have been implicated in the pathogenesis of severe neurological and systemic manifestations in COVID-19 patients, largely through mitochondrial dysfunction and cytokine-mediated oxidative injury [50]. By analogy, the severe neurological signs observed in the cats in our study may reflect a similar imbalance, wherein excessive ROS production overwhelms depleted antioxidant systems, including SOD. This oxidative dysregulation could potentially exacerbate neuronal damage and clinical deterioration in feline infectious peritonitis. Further studies involving larger populations are warranted to elucidate this relationship and validate the role of oxidative stress in the neuropathogenesis of FIP.

Oxidative stress and damage to cellular components may be mitigated through antioxidant therapy. Various antioxidants and their supplements have been shown to be effective against different neurological diseases [51]. Future studies in cats with FIP could assess the potential benefits of antioxidant therapy in disease management.

In our study, cerebrospinal fluid (CSF) total protein concentrations in cats with neurological FIP were found to be elevated compared to established reference intervals. This finding is consistent with the results of Crawford et al.[52], who reported markedly increased CSF total protein levels in all 11 affected cats, with a mean of 940 mg/dL. These data support the presence of a strong inflammatory response within the central nervous system in feline neurological FIP. Similarly, Rand et al.[53] reported a mean CSF total protein concentration of 368 mg/dL, which is still markedly elevated when compared to healthy cats. On the other hand, Steinberg et al.[54] reported substantially lower protein concentrations, with a mean of only 18 mg/dL. These variations among studies may be attributed to differences in disease stage at the time of sampling, the degree or distribution of CNS lesions (focal vs. diffuse) [55], or the site of CSF collection [56]. It is also known that lumbar CSF samples tend to have higher protein concentrations and lower white blood cell counts compared to cerebellomedullary cistern samples [56]. Therefore, both biological and methodological factors may account for the discrepancies observed across different studies.

This study has several limitations. First, CSF 8-OHdG and SOD levels were measured at only a single time point during the disease. Conducting a longitudinal study that assesses these biomarkers at multiple stages following FIP infection would be essential to better understand their temporal dynamics and evaluate their prognostic value in later stages of the disease. Furthermore, whether this oxidative perturbation in DNA metabolism plays a role in initiating or sustaining neuronal cell death in neurological FIP remains to be determined. If so, pharmacological interventions targeting DNA modifications could potentially offer a novel therapeutic approach for affected cats.

Another limitation of this study is that it focused solely on cerebrospinal fluid (CSF) concentrations of 8-OHdG. However, numerous previous studies -both in human and veterinary medicine- have evaluated 8-OHdG levels in serum or plasma across a variety of diseases [57-61]. These studies have demonstrated that elevated circulating 8-OHdG levels are associated with oxidative stress-related pathologies, such as neurodegenerative diseases [60], cancers [59], and chronic inflammatory conditions [57,62]. Particularly in clinical settings where CSF collection is not feasible, measuring serum or plasma 8-OHdG levels could offer a less invasive and more accessible diagnostic alternative.

Additionally, a notable limitation of this study is the relatively small sample size. However, enrolling cats with confirmed neurological FIP that were also negative for both FeLV and FIV proved particularly difficult. Obtaining informed consent for necropsy procedures was also challenging. Further constraints included the narrow postmortem time window required for cerebrospinal fluid collection, rapid clinical deterioration in neurological cases, and logistical limitations encountered during sample collection. Despite these difficulties, strict diagnostic criteria and careful case selection were employed to ensure the scientific reliability of the results. Although limited in number, the data provide valuable preliminary insights into oxidative stress biomarkers in feline neurological FIP. In feline infectious peritonitis, significantly elevated CSF levels of 8-OHdG, coupled with markedly reduced concentrations of SOD, indicate an increased state of oxidative damage in DNA and a compromised antioxidant defense mechanism. This study highlights the role of oxidative stress and inflammatory processes in the pathophysiology of neurological FIP, wherein augmented oxidative damage and metabolic disturbances may contribute to neuronal injury.

In conclusion, this study demonstrated significantly increased CSF levels of 8-OHdG and decreased concentrations of superoxide dismutase (SOD) in cats with neurological FIP. These findings suggest that oxidative stress and impaired antioxidant defense mechanisms may be involved in the neuropathogenesis of the disease. Within the scope of this study, the measurement of 8-OHdG and SOD in CSF contributes to the understanding of the oxidative processes associated with neurological FIP. Further research is warranted to better define the relevance of these biomarkers in the clinical evaluation of affected cats.

Declarations

Availability of Data and Materials: The data supporting this study's findings are available from the corresponding author (İ. Baştan) upon reasonable request.

Funding Support: None

Conflict of Interest: The authors declared that there is no conflict of interest.

Declaration of Generative Artificial Intelligence (AI): The authors declare that the article, tables and figures were not written/created by AI and AI-assisted Technologies.

Author Contributions: I.B. and D.I.I. conceived and designed the study. I.B., D.I.I. and S.H.E. performed the sample collection. Y.K.K. and T.S. conducted the CSF analyses. Y.K.K. and S.H.E. performed the RNA extraction and real-time RT-PCR for FCoV detection. A.S.T. performed the necropsies and conducted the histopathological examinations. I.B. and Y.K.K. drafted the manuscript. All authors reviewed and approved the final version of the manuscript.

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