Assessment of oxidative stress biomarkers in felines infected with calicivirus

ABSTRACT

Feline Calicivirus (FCV) affects cats, causing respiratory and oral issues. Oxidative stress is a key aspect in FCV pathology, stemming from imbalances between reactive oxygen species (ROS) and antioxidant defenses. Given cats' heightened sensitivity to oxidative stress, this study aims to explore its presence in FCV-afflicted felines via serum markers. A total of 20 Plasma samples from the Control Group cat and patient group (n=10 each) were obtained and the patient group was confirmed using RT-PCR. Additionally, the presence of Plasma markers (Malondialdehyde: MDA, Superoxide Dismutase: SOD, Catalase, Glutathione Peroxidase: GPx, and Total Antioxidant Capacity: TAC) for oxidative stress were assessed using the ELISA kit. Finally, data analyses and visual representations were executed using Python. Distinct variations in oxidative stress markers among feline cohorts were observed. Patient SOD and GPx levels were 39.73 u/L and 75.63 u/L, respectively, while controls showed 36.41 u/L and 218.48 u/L (p-values: 0.05, 0.017). CAT and MDA values in patients were 3.7 u/L and 9.85 nmol, respectively, contrasting with 11.81 u/L and 4.17 nmol in controls (p-values: 0.002, 0.050), while TAC levels showed minimal differences. The study's results revealed distinct changes in oxidative markers like SOD, GPx, and MDA compared to healthy cats. The slight increase in SOD and decreased GPx activity suggests heightened oxidative stress. Furthermore, these findings highlight potential oxidative disruptions in FCV-infected cats, emphasizing the need for further investigation and potential therapeutic strategies. Additionally, exploring potential therapeutic interventions, like antioxidant supplementation, may open avenues for improved disease management strategies in affected felines.

Keywords: Oxidative stress, Feline calicivirus, Malondialdehyde, Superoxide dismutase, ELISA.

1. Introduction

Feline Calicivirus (FCV) frequently emerges as a primary concern in cats, manifesting as respiratory challenges or oral ulcers and usually resulting in multiple health complications (1). This virus is notably associated with oral region sores, particularly affecting the tongue and roof of the mouth, and might also be accompanied by upper respiratory tract subtle signs. FCV colonization can lead to pronounced pulmonary complications within juveniles and infrequently in adult felines (2). This virus belongs to the Vesivirus genus within the Caliciviridae family and is characterized as a single-stranded, positive-sense RNA virus without an envelope. Spanning 7.5 kb, the FCV genetic material comprises three interrelated open reading frames (ORF). The first ORF encodes non-structural proteins, while the second is responsible for the VP1 major capsid protein and the third for the VP2 minor capsid protein. Due to its RNA genome, it is anticipated that FCV would exhibit significant genomic variability, with a range of 1.3 × 10^-2 to 2.6 × 10^-2 substitutions per nucleotide (3). This tendency stems from the absence of a proofreading mechanism, commonly seen in viral RNA-dependent RNA polymerases, leading to reduced fidelity. Such propensity for replication with errors grants FCV enhanced adaptability, enabling it to adapt to novel environmental settings. This adaptability results in contemporary challenges linked to FCV, including encompassing the complexities in selecting typical strains for vaccination, the occurrence of continuously infected felines, and the rise of exceptionally virulent FCV strains (4). To address complications induced by FCV, a comprehensive assessment of the virus's pathogenic attributes is imperative. Therefore, this study aims to enhance our understanding of FCV pathogenesis.

FCV primarily replicates itself in respiratory tissues; however, it can also transmit to other tissues such as visceral tissues, feces, and occasionally in urine. This virus does not possess a specific cap structure and replicates itself within intercellular membranes through a minus-strand RNA intermediate (5). According to a study by Alessandro Natoni et al., FCV can trigger the mitochondrial apoptosis pathway by inducing the translocation of phosphatidylserine to the host cell's outer

membrane (6). A further understanding of the molecular mechanisms and pathways of this infection will help us comprehend the pathogenicity of this virus. As a result, this study aims to identify the presence of oxidative stress, one of the important cellular pathways during FCV infection, to enhance our understanding of FCV pathogenesis.

Oxidative stress characterizes a disparity between elevated concentrations of reactive oxygen species (ROS) and diminished antioxidant defense activity. Excessive oxidative stress can lead to cellular damage and may result in tissue degradation. Although, ROS plays an indispensable role in optimal cellular operations, notably energy generation by the mitochondria, augmented oxidative stress has been implicated in natural processes like aging and physical exertion, as well as in various disease states encompassing oncological conditions, neurodegenerative disorders, cardiovascular ailments, diabetes, inflammatory conditions, and toxin-induced disturbances (7). Felines appear to display heightened vulnerability to oxidative stress and its subsequent harm. This predisposition might be shaped by their distinct spleen anatomy (8,9). Instances of oxidative stress have been observed in several disorders affecting this species, including diabetes mellitus (10), chronic kidney disease (11), and feline immunodeficiency virus (FIV) infections (12). To identify the presence of oxidative stress, it is crucial to select the appropriate biomarkers that indicate its existence. Several studies have highlighted the significance of three key enzymes in oxidative stress: superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) (13). Moreover, many molecules undertake the oxidation process, particularly lipids, which are prone to oxidation due to their molecular composition rich in reactive double bonds (14). Lipid peroxidation stands as a central element in the realm of oxidative stress, among the extensively researched indicators of lipid peroxidation are isoprostanes (IsoPs) and malondialdehyde (MDA) (15).

The total antioxidant capacity (TAC) is another indicator in serum, reflects the collective influence of all antioxidants found in the bloodstream. It offers a comprehensive metric of oxidative conditions.

An optimal superoxide production serves as a beneficial metabolite, assuming pivotal roles in cellular processes such as division, while also acting as a countermeasure against lipid peroxidation. However, excessive production of this radical can lead to lipid peroxidation initiation, protein impairment, and DNA damage. This cascade of events can result in cellular dysfunction and eventual demise through apoptosis or necrosis. The dual-edged nature of these properties makes it challenging to reinstate the ideal equilibrium between superoxide and SOD, especially when disrupted by trauma, illness, or the aging process (16). The intracellular antioxidant system encompasses both enzymatic and non-enzymatic components. As mentioned before, the enzymatic elements include SOD, CAT, and GPx, while glutathione stands out as a non-enzymatic antioxidant (AOX) (15). SOD facilitates the dismutation of O2•-, resulting in O2 and H2O2 as byproducts. Meanwhile, CAT and GPx collaborate to break down H2O2 into H2O and O2. Conversely, when reactive species (RS) are excessively generated and exceed AOX's neutralizing capacity, the balance tilts towards oxidants, leading to the onset of oxidative stress (17).

Assessing biomarkers related to oxidative stress offers a holistic perspective on the presence of reactive oxygen species (ROS) and the efficacy of the antioxidant (AOX) system. The aim of this study is to ascertain the existence of oxidative stress in felines with FCV by measuring serum oxidative biomarkers. Deepening our insights into this ailment and its associated oxidative stress patterns may pave the way for pinpointing treatment strategies leveraging antioxidants or agents that temper oxidative stress.

2. Materials and Methods

2.1. Sample Collection

Plasma specimens utilized in this research were meticulously procured from Nikan Pet Hospital in Tehran Province. The samples were then organized into two specific groups for a detailed comparative study. The first, known as the Control Group, included plasma samples extracted from

10 cats that had been brought in for regular health screenings. Intriguingly, these felines, despite their varied backgrounds and age groups, demonstrated no discernible clinical anomalies upon comprehensive physical evaluations.

On the other hand, the Patient Group presented a contrasting scenario. This group comprised plasma samples from another set of 10 cats. A distinguishing characteristic of these cats was that each one of them had tested positive for FCV when subjected to the RT-PCR diagnostic method. Further compounding their health status was a universally shared clinical sign amongst them - the presence of oral ulcers, hinting at the virulence and symptomatic manifestation of the infection. The ages of all feline subjects in the study ranged from 8 to 24 months.

Once collected, all serum samples underwent stringent storage protocols. They were methodically preserved at an ultra-low temperature of -80°C. This ensured the stability and integrity of the samples, making them primed for the subsequent laboratory analyses. These imminent tests were designed with precision to target and quantify specific markers, namely MDA, SOD, Catalase, GPx, and TAC. Such markers are integral to understanding the oxidative stress levels and overall health status of the subjects.

2.2 RT-PCR

During the acquisition of plasma samples from felines showing potential clinical signs, concurrent rectal and mucosal specimens were obtained. These specimens underwent an RT-PCR analysis targeting FCV, utilizing the primers delineated by Kim et al (18). Subsequent to evaluation, samples returning positive results were incorporated into our designated "Patient Group."

2.3. ELISA

For the assessment of MDA, SOD, GPx, CAT, and TAC levels, we employed the ZellBio[1] ELISA kit. Throughout the assay procedures, strict adherence to the manufacturer's guidelines was maintained. The outcomes for all parameters, with the exception of MDA, were determined using an ELISA reader. MDA levels, indicative of lipid peroxidation, were measured photometrically. SOD activity represents the conversion of O2•- to H2O2 and O2. Both CAT and GPx play pivotal roles in the decomposition of H2O2 into H2O and oxygen. Furthermore, TAC served as an index reflecting the overall status of the AOX system.

2.4. Statistical Analysis

All statistical analyses were conducted using SPSS. The mean, maximum, minimum and P values of each enzyme activity were calculated utilizing T-Test methods. To visually represent the data, graphs were generated using the Matplotlib library in python. The complete coding script, detailing the procedures undertaken for the graph, can be accessed on the GitHub repository of Sajed Sarabandi.

2.5. Ethical Considerations

The study protocol, including sample collection and analyses, received formal approval from the Ethics Committee of the Karaj Islamic Azad University and the reference code is IR.IAU.K.REC.1401.153

3. Results

Our investigation delved into the oxidative stress parameters across two distinct groups: patients and controls. We identified notable variances in these parameters, which may offer profound insights into the underlying biochemical pathways associated with the disease conditions.

In our research based on (Figure 1), the observed average levels for the Superoxide Dismutase (SOD) enzyme in the afflicted cohort stood at 39.73 u/L, demonstrating a variability denoted by a standard

[1] https://zellbio.eu/

error of 5.11. In contrast, our healthy subjects recorded an average SOD concentration of 36.41 u/L, characterized by a 2.33 standard error. This yielded a statistical significance indexed at p=0.05. Transitioning to the Glutathione Peroxidase (GPx) enzyme, the data for the affected group showcased a median value of 75.63 u/L with a fluctuation encapsulated within a 14.86 standard error. The unafflicted subjects, however, registered a prominent GPx mean value of 218.48 u/L and an oscillation of 34.6, culminating in a noteworthy p-value of 0.017. The Catalase (CAT) enzymatic activity in the affected sample settled at an average of 3.7 u/L with a standard fluctuation of 0.43, whereas their healthy counterparts presented a CAT mean of 11.81 u/L and a variation of 1.46. The resulting p-value was a pronounced 0.002. Diving into the Malondialdehyde (MDA) metrics, the central tendency for our patient pool was discerned at 9.85 nmol, exhibiting a tight spread of 0.98. The baseline group, meanwhile, projected an MDA mean of 4.17 nmol with an accompanying spread of 0.68, leading to a consequential p-value of 0.050. Finally, the Total Antioxidant Capacity (TAC) exhibited an average value of 1.35 µmol/L, with a nominal standard error of 0.02.

In summary, our findings underscored distinct intergroup variations in several oxidative stress parameters, potentially elucidating the biochemical intricacies associated with the examined conditions.

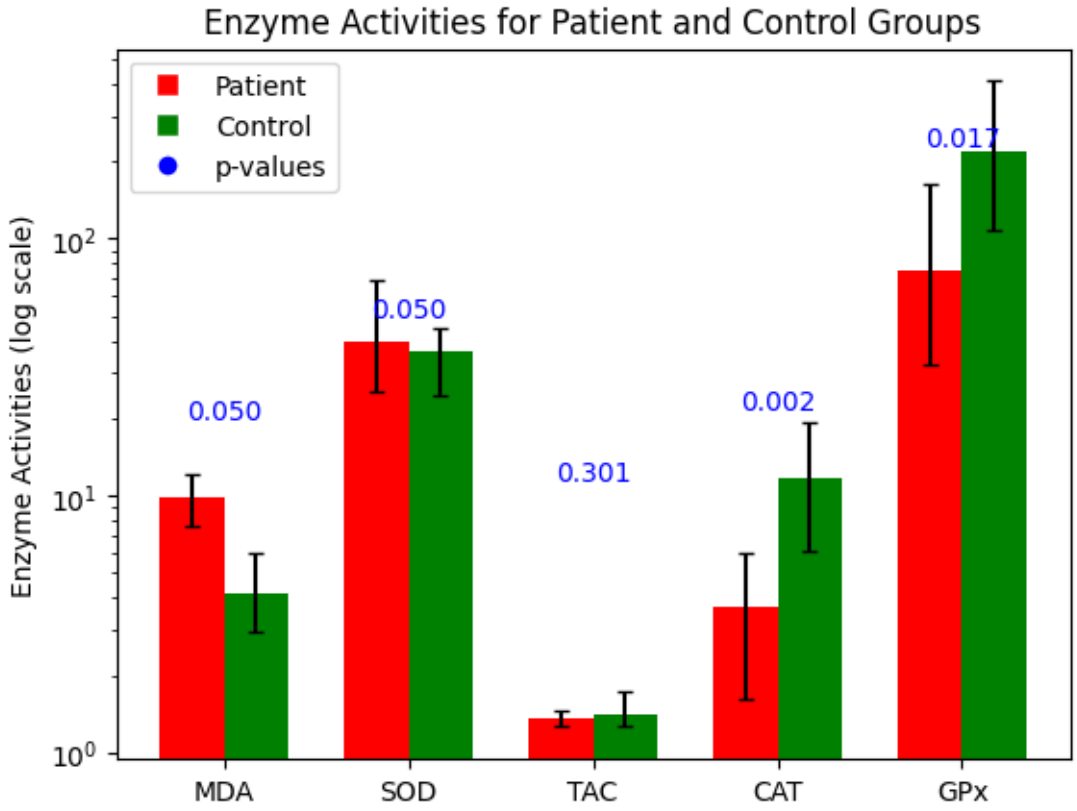

Figure 1. This figure illustrates the logarithmic activities of each enzyme along with their corresponding estimated p-values. The red color represents the patient group, while the green color represents the control group.

4. Discussion

Oxidative stress is disequilibrium favoring oxidants over antioxidants, which may result in cellular harm (19). Oxidants are typically produced during aerobic metabolism, and their production can increase under certain pathological conditions. Normally, a careful equilibrium is maintained by a complex network of antioxidants. These antioxidant mechanisms can adapt to changing needs to a certain extent (20, 21).

The intricate relationship between oxidative stress and disease pathogenesis has gained momentum in veterinary research over recent years. In the study by Ho and colleagues, enzymatic antioxidant activities, namely CAT, GPx, and SOD, were meticulously measured in plasma to gauge the antioxidant potential (15). Therefore, the present study contributes to this growing body of evidence

by discerning the differences in key oxidative stress parameters, namely SOD, GPx, CAT, and MDA TAC between two feline groups, including patients affected by FCV and a healthy control cohort. Figure 2 demonstrates the relationship between mentioned enzymes and oxidative stress.

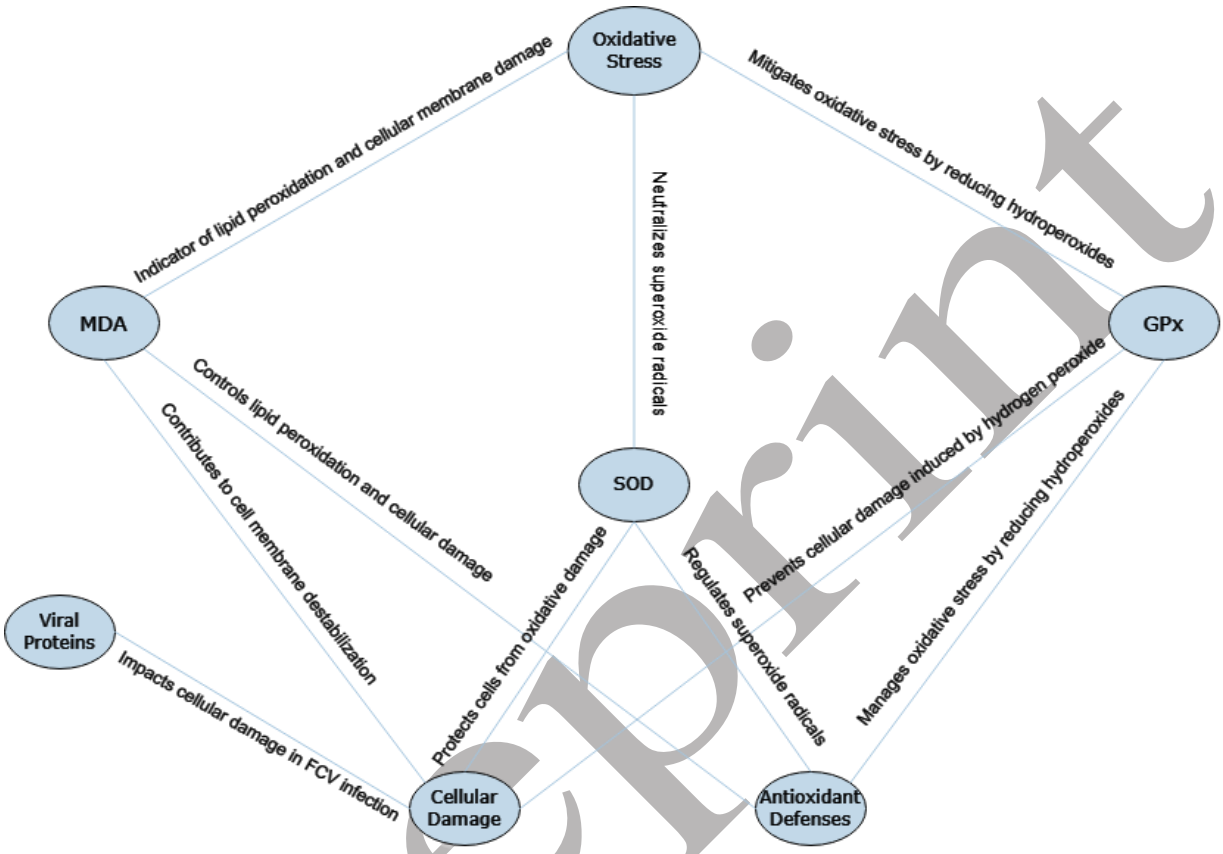

Figure 2. This diagram illustrates the connection between the biomarker and oxidative stress

The SOD plays a crucial role in neutralizing superoxide radicals, serving as the primary defense against oxygen-derived free radicals (ROS). In response to elevated ROS levels, SOD catalyzes their conversion to hydrogen peroxide (H2O2), which, in turn, protects cells from oxidative damage. According to our findings, there is an observed increase in SOD levels within the patient group compared to the control group. The movement of superoxide is somewhat restricted within cellular environments, primarily because it struggles to permeate cell membranes. Furthermore, its short-lived presence indicates that its effects are likely confined to its immediate region of formation (22,23).

Glutathione Peroxidase (GPx) serves as the second line of defense against oxidative injury. Typically, GPx aids in mitigating oxidative stress by converting H2O2 and organic hydroperoxides into water and their respective alcohols (24). Increased activity of reactive oxygen species (ROS) resulted in a high amount of H2O2. In this case, both GPx and catalase (CAT) work to detoxify the produced H2O2 to protect the cell. However, GPx requires glutathione as a cofactor, and in the absence of glutathione, it cannot efficiently detoxify H2O2. Our findings revealed a significant decrease in GPx activity in the patient cohort compared to the controls. This reduction may occur due to a decrease in glutathione levels. A diminished GPx and CAT activity can leave cells vulnerable to hydrogen peroxide, which can further disintegrate to form highly reactive hydroxyl radicals, escalating oxidative stress. The observed decrease in GPx activity in FCV-infected cats underscores a crucial aspect of viral infections. Viruses often manipulate host cell machinery, including antioxidant defenses, to create an environment conducive to their replication. Consequently, the diminished GPx activity might stem from the virus's interference with the host's antioxidant systems. This finding emphasizes the need for antiviral strategies that not only target the virus directly but also bolster the host's antioxidative defenses.

Additional evidence of oxidative imbalance emerged upon analyzing MDA levels. MDA, a low-molecular-weight tri-carbon aldehyde, stems from the degradation of polyunsaturated fatty acids in biological membranes induced by free radicals. The insufficient activity of GPx and CAT, coupled with an increase in superoxide radicals, leads to elevated lipid peroxidation. Evaluating MDA levels serves as a dependable indicator of lipid peroxidation in biological specimens, given that it is a significant product of this mechanism (25). The nearly doubled MDA concentration in the patient group, compared to controls, unveils a scenario of heightened cellular membrane damage, possibly instigated by reactive oxygen species (26, 27). Moreover, the elevated MDA levels in FCV-infected cats highlight the severity of cellular damage induced by oxidative stress. MDA, a byproduct of lipid peroxidation, is not merely a marker but also an active contributor to cell membrane destabilization.

This suggests a potential vicious cycle where viral-induced oxidative stress damages cellular membranes, leading to further oxidative stress. Interrupting this cycle could be a promising avenue for therapeutic interventions. Exploring antioxidants or compounds that specifically target lipid peroxidation might present new possibilities for mitigating FCV-induced oxidative stress and its consequences. Though the difference bordered statistical significance, from a biological perspective, such an elevation cannot be overlooked.

Finally, Total Antioxidant Capacity (TAC) offers a comprehensive perspective on the collective activity of both enzymatic and non-enzymatic antioxidants. In the presence of oxidative stress, it has been speculated that the number of antioxidants may decrease as they attach to oxidative radicals to detoxify them. It is crucial to note that TAC is not only influenced by the quantity of oxidative radicals; it also changes based on feeding conditions and nutritional intake. According to our analysis, TAC decreased in the patient group, indicating an increase in oxidant radicals within this group.

Considering the importance of these findings, further studies should delve into the intricate mechanisms of oxidative stress in FCV infections. Investigating how specific viral proteins modulate oxidative stress-related pathways could reveal novel therapeutic targets. Additionally, longitudinal studies monitoring these oxidative stress markers during different stages of FCV infection could provide valuable insights into the disease's progression and severity.

In conclusion, our findings accentuate a potential oxidative imbalance in cats infected with FCV, hinting at oxidative stress as a possible player in the etiology or progression of the disease. This oxidative stress, marked by disrupted SOD, GPx, CAT, and MDA levels, showcases the profound impact of FCV infection on feline physiology. These alterations underscore the need for a multifaceted approach in treating FCV, integrating antiviral strategies to combat the virus alongside antioxidative therapies to alleviate the oxidative burden on feline cells. While our results provide valuable insights, they also pave the way for more in-depth investigations. A deeper dive into the

mechanisms causing these alterations, perhaps through molecular and genetic studies, could offer a more comprehensive understanding. Additionally, exploring potential therapeutic interventions, like antioxidant supplementation, may open avenues for improved disease management strategies in affected felines.

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Authors' Contributions

HP designed this study. KF and SS drafted manuscript. PY and KF did the laboratory tests. PY preformed the statistical analysis. SS prepared the figure. HP and KF edited the final manuscript. HP, KF, PY, and SS read and approved the final manuscript.

Availability of data and materials

The data sets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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