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Research Article

Determination of Thiol/Disulfide Homeostasis and Oxidative Stress Index in Sheeppox Virus

Sinan ADIGÜZEL[1], Oğuz MERHAN[2*]

[1]*Kafkas University, Institute of Health Sciences, Kars, TÜRKİYE.*
[2]*Kafkas University, Faculty of Veterinary, Department of Biochemistry, Kars, TÜRKİYE.*

ABSTRACT

The study aimed to evaluate the balance of thiol/disulfide homeostasis and oxidative stress markers in sheep infected with pox virus and to explore their potential diagnostic value. 1-2 years old Morkaraman breed sheep (20 infected with pox virus, 10 healthy) were used in the study. Analyses of total thiol, native thiol, total antioxidant capacity, and total oxidant capacity were conducted on serum samples collected from the sheep's jugular vein using tubes without anticoagulant. Comparison between the pox virus-infected group and healthy sheep revealed a significant decrease in total thiol and native thiol levels, while disulfide, disulfide/native thiol, and disulfide/total thiol levels increased, though these changes were not statistically significant. In addition, it was determined that serum total oxidant capacity and oxidative stress index levels increased, while total antioxidant capacity level decreased. In conclusion, the study's findings indicate that the disease induces oxidative stress. The use of oxidative stress markers, particularly those related to thiol/disulfide homeostasis, may provide insights into the pathogenesis of sheeppox and serve as an additional diagnostic tool.

Keywords: *Oxidative stress index, sheeppox, thiol/disulfide homeostasis.*

Koyun Çiçek Hastalığında Tiyol/Disülfid Homeostazı ve Oksitadif Stres İndeksinin Belirlenmesi

ÖZET

Çalışmadaki amacımız çiçek virüsü ile enfekte koyunlarda tiyol/disülfid homeostazisi ve oksidatif stres parametreleri düzeylerinin belirlenmesi ve bunların diagnostik önemlerinin araştırılmasıdır. Çalışmada 1-2 yaşlı Morkaraman ırkı koyunlar (20 adet çiçek virüsü ile enfekte, 10 adet sağlıklı) kullanıldı. Koyunların *Vena jugularis*'inden antikoagulansız tüplere alınan kan örneklerinden elde edilen serumlarda total tiyol, natif tiyol, total antioksidan kapasite ve total oksidan kapasite analizleri yapıldı. Çiçek virüsü ile enfekte grup ile sağlıklı koyunlar karşılaştırıldığında total tiyol ve natif tiyol düzeylerinin anlamlı olarak azaldığı; disülfid, disülfid/natif tiyol ve disülfid/total tiyol düzeyleri ise artmakla beraber istatistiksel olarak anlamsız olduğu belirlendi. Bunun yanı sıra serum total oksidan kapasite ve oksidatif stres indeksi düzeylerinin arttığı, total antioksidan kapasite düzeyinin ise azaldığı saptandı. Sonuç olarak, çalışmada elde edilen bulgular hastalığın oksidatif strese neden olduğu, oksidatif stres belirteçlerinden özellikle de tiyol/disülfid homeostazis parametrelerinin kullanımı çiçek hastalığının patogenezine katkı sağlayacağı ve teşhisi için yardımcı parametre olabileceği düşünülmektedir.

Anahtar kelimeler: *Koyun çiçeği, oksidatif stres indeksi, tiyol/disülfid homeostazı.*

***Corresponding author:** Oğuz MERHAN, Kafkas University, Faculty of Veterinary, Department of Biochemistry, Kars, TÜRKİYE.*
oguzmerhan@hotmail.com
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Introduction

Sheeppox virus is a zoonotic disease that affects sheep, goats and humans. The disease still maintains its importance as it is commonly seen in sheep and goats in many parts of the world. In addition to abortion, death, loss of productivity and treatment costs resulting from the disease, the implementation of import bans on live animals and animal products in countries where it is seen negatively affects the economy of small livestock farming (Bowden, 2008; Plowright, 2012). The disease, caused by a virus belonging to the Capripox genus in the Poxvirus family. While the severity of the disease can be influenced by factors such as the animal's age, sex, breed, and physiological condition, it tends to be particularly severe in young lambs (Bhanuprakash et al., 2006).

Thiols are a very important antioxidant in preventing damage caused by oxidative stress and protects the cell against oxidative stress. It appears that the thiol status changes in various diseases and that thiol/disulfide homeostasis is very important in the pathogenesis of diseases. Consequently, assessing thiol/disulfide homeostasis can offer valuable insights into a range of physiological and pathological processes. It is known that oxidative stress occurs in situations such as infection and stress, and as a result, the total oxidant/antioxidant capacity may change and can be used as a marker. In light of this information, researching early markers of inflammation in human and veterinary medicine has gained importance in recent years (Erel and Neselioglu, 2014; Kükürt et al., 2021). When the balance between antioxidants and oxidants in the organism is disrupted for various reasons, free reactive radicals appear (Pizzino et al., 2017). Free radicals can cause damage by affecting the basic building blocks of the body such as lipids, carbohydrates and proteins. If the organism's defence mechanism cannot repair the damage, the cells cannot perform their functions, and the immune system weakens and the severity of the infection increases. As a result, apoptosis may occur in cells (Özcan et al., 2015).

It has been reported that free radicals play a role in the formation of diseases (Akaike, 2001), and that the sheeppox virus affects membrane phospholipids by causing an increase in free radicals and, accordingly, causes damage to cells and tissues (İssi et al., 2008). Therefore, our aim in the study is to determine the levels of thiol/disulfide homeostasis and oxidative stress parameters in sheep infected with pox virus and to investigate their diagnostic importance.

Material and Methods

This study was started after receiving the ethics committee approval of Kafkas University Animal Experiments Local Ethics Committee (KAÜ-HADYEK) dated 21.11.2022 and coded 2022/178. In the study, 20 sheep naturally infected with smallpox virus (showing clinical symptoms such as fever, loss of appetite, rhinitis, respiratory problems, smallpox lesions in different parts of the mucosa and skin such as under the tail, eyelids, armpits, between the hind legs, etc.) and 10 healthy sheep (1-2 year old Morkaraman breeds) were used. Before starting the study, the infection was confirmed by taking samples from the lesions in these sheep and isolating and identifying the poxvirus in the research laboratory (Erzurum Veterinary Control Institute-Turkey). The samples taken into the 10 mL tubes without anticoagulant were centrifuged at 3000 rpm for 15 minutes. The serum samples were stored at -20 °C until the analysis would be made.

Total thiol, native thiol, total antioxidant capacity (TAC) and total oxidant capacity (TOC) (Rel Assay Diagnostics, Turkey) in the obtained serum samples were measured colorimetrically (Epoch, Biotek, USA) using commercial test kit. A novel automatic and spectrophotometric technique established by Erel and Neselioglu (2014) was used to determine the thiol/disulphide concentrations. The principle of this method is based on the degradation of dynamic disulphide bonds (-S-S-) to functional thiol groups (-SH) with a sodium borohydride ($NaBH_4$) solution. The remaining $NaBH_4$ residue was totally removed by formaldehyde. Thus, this inhibited extra reduction of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) along with any disulphide bonds resulting from the reaction with DTNB. The following reaction with the DTNB-modified Ellman reagent was used to detect the amount of total thiol. Disulfide = (Total thiol-Native thiol)/2, Disulfide/Native Thiol (%) = (Disulfide x 100)/Native thiol, Disulfide/Total Thiol (%) = (Disulfide x 100)/Total thiol and Native Thiol/Total Thiol (%) = (Native thiol x 100)/Total thiol (Erel and Neselioglu, 2014).

TAC measurement is based on the principle that antioxidant substances present in the sample react with the blue-green ABTS [2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)] radical, causing a decrease or loss of color in the color of the compound. TOC measurement is based on the principle that oxidants present in the sample oxidize the Fe^{+2}-o-dianisidine complex to ferric (Fe^{+3}) ion. Fe^{+3} forms a colored complex with xylenol orange in acidic medium. It was calculated with the formula oxidative stress index (OSI) = (TOC/10xTAC) (Karababa et al., 2013).

Statistical Analysis

The study data were analyzed using the SPSS software package (SPSS 20.0, IBM SPSS Statistics®, Chicago, IL, USA). The distribution of the data obtained from the groups were shown as normal distribution according to the Kolmogorov-Smirnov test. An Independent Samples T-test was employed to compare the groups. $P \leq 0.05$ value was statistically considered significant.

Results

When the sheeppox virus-infected group was compared with healthy sheep, it was determined that total thiol and native thiol ($P<0.01$) levels decreased statistically significantly, while disulfide, disulfide/native thiol and disulfide/total thiol levels increased insignificantly. In

addition, although the native thiol/total thiol level decreased, it was determined to be statistically insignificant (P>0.05) (Table 1). It was determined that serum TOC and OSI levels increased, while TAC (P<0.01) levels decreased statistically significantly (Table 2).

ed in cattle with endometritis that native thiol and total thiol levels decreased statistically significantly compared to the control group, while disulfide, disulfide/native thiol, disulfide/total thiol and native thiol/total thiol levels were statistically insignificant. In this study, when the

Table 1. Thiol/disulfide homeostasis parameters in clinically healthy and sheeppox virus (X±SEM)

Parameters	Control	Infected	P
Total Thiol (µmol/L)	317.31±20.66	243.09±9.13	0.006
Native Thiol (µmol/L)	267.90±19.30	187.21±9.22	0.002
Disulfide (µmol/L)	24.71±3.05	27.93±3.76	0.509
Disulfide/Native Thiol (%)	9.60±1.27	17.17±3.39	0.135
Disulfide/Total Thiol (%)	7.87±0.93	11.37±1.49	0.127
Native Thiol/Total Thiol (%)	84.25±1.87	77.25±2.99	0.126

Table 2. TAC, TOC and OSI parameters in clinically healthy and sheeppox virüs (X±SEM)

Parameters	Control	Infected	P
TAC (mmol Trolox Equiv/L)	1.42±0.07	1.15±0.06	0.007
TOC (µmol H_2O_2 Equiv/L)	5.61±0.26	7.78±0.52	0.008
OSI (Arbitrary Unit)	0.40±0.02	0.72±0.07	0.004

TAC: Total Antioxidant Capacity, TOC: Total Oxidant Capacity, OSI: Oxidative Stress Index

Discussion

Albumin contains thiol sulfhydryl groups, which are proteins and low molecular weight thiols. This sulfhydryl group plays a crucial role in mitigating oxidative stress. Thiol groups found in sulfur-containing amino acids, such as methionine and cysteine, are primary targets for reactive oxygen species (Erel and Neselioglu, 2014). Thiol groups oxidized by the effect of reactive oxygen species form disulfide bonds (Ates et al., 2016). Determination of thiol/disulfide homeostasis is an indicator of free radical formation (Aksoy and Kirmit, 2020). Free radicals cause deterioration in the functions of thiol-related enzymes and thiol/disulfide homeostasis in the cellular environment (Erel and Neselioglu, 2014).

Thiol/disulfide homeostasis, a new biomarker for oxidative stress, was determined in veterinary medicine; toxoplasmosis (Aydın et al., 2023), gastrointestinal nematodes (Schmidt et al., 2021), foot diseases (Deveci and Erdal, 2022), babesiosis (Tarhan et al., 2023), canine distemper (Değirmençay et al., 2021), sarcoptic mange in sheep (Çamkerten et al., 2019) in calves with neonatal diarrhea (Terzi et al., 2023), dehorning in cattle (Erdoğan et al., 2019), and young cattle with pneumonia (Ertaş et al., 2023). It has been reported that total thiol and native thiol significantly decreased, while disulfide, disulfide/native thiol and disulfide/total thiol increased in sheep with toxoplasmosis (Aydın et al., 2023). In calves with neonatal diarrhea, total thiol and native thiol levels were lower, and disulfide and disulfide/native thiol levels were higher in the diarrheal group (Terzi et al., 2023). Additionally, Emre et al. (2021) reported in a study conducted sheeppox virus infected group and healthy sheep were compared, native thiol and total thiol levels decreased statistically significantly, disulfide, disulfide/native thiol and disulfide/total thiol levels increased but were statistically insignificant, as well as native thiol levels. Although the native thiol/total thiol level decreased, it was determined to be statistically insignificant. The reason for the change in homeostasis can be explained by the severity of thiol oxidation and oxidative stress.

Under physiological conditions, oxidants and antioxidants are in balance. However, in case of disease, this balance is eliminated, and oxidative stress occurs. Antioxidants prevent the formation of free radicals and eliminate the oxidants that form. Although oxidative stress level is determined by many methods, it is reported that measuring total oxidant or antioxidant capacity is more useful in studies since the methods that measure molecules one by one are both expensive and time-consuming (Erel, 2004; Erel, 2005). Oxidative stress is important in the formation of inflammatory conditions, and studies have reported that oxidative stress occurs in infectious diseases (Bozukluhan et al., 2017; Merhan et al., 2017; Merhan et al., 2020). In their study on sheep infected with ecthyma, Deveci et al. (2017) reported that TOC and OSI levels increased, indicating oxidative stress. This stress resulted from an imbalance between pro-oxidant and antioxidant molecules, driven by rising oxidant levels. It was reported that oxidative stress occurred, and the level of antioxidants decreased in sheeppox-infected sheep (Kirmizigül et al., 2016). Additionally, in cattle infected with lumpy skin, El Mandrawy and Alam (2018)

reported that the MDA level, increased and the GSH level decreased. In the study, it was determined that TOC and OSI levels increased, and TAC levels decreased in sheep infected with sheeppox. It is thought that the change in oxidative stress index values may be due to the increase in free radicals formed against the virus in host cells.

Conclusion

As a result, it is thought that the findings obtained in the study cause oxidative stress in the disease and the use of thiol/disulfide homeostasis markers will contribute to the pathogenesis of sheeppox disease and may be useful in studies to be conducted for its diagnosis.

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Conflict of interest

The authors declare that there is no conflict of interest between them.

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