Investigation of some acute phase proteins and antioxidant/oxidant system in infected sheep with bluetongue virus disease

Research Article

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ABSTRACT

In this study, it was aimed to determine the level of some acute phase proteins and oxidative stress in sheep infected with bluetongue virus disease. Twenty five bluetongue virus-infected and 10 healthy sheep were used. Blood samples from V. jugularis of animals were taken into tubes without anticoagulant. Total antioxidant (TAC)/oxidant capacity (TOC), haptoglobin, serum amyloid A (SAA), ceruloplasmin and albumin levels were determined colorimetrically. Oxidative stress index (OSI) was calculated using the formula. As a result of the analysis, when sheep infected with bluetongue virus disease and healthy sheep were compared, it was determined that SAA, TOC and OSI concentrations increased, albumin and TAC values decreased. However haptoglobin and ceruloplasmin levels increased but were statistically insignificant. In conclusion, it was concluded that oxidative stress occurs in sheep infected with bluetongue virus disease and that acute phase proteins haptoglobin, SAA and ceruloplasmin can be used as inflammation markers.

Keywords: Acute phase proteins, bluetongue virus disease, oxidative stress index (OSI), sheep

INTRODUCTION

Bluetongue disease caused by Orbivirus is a viral disease of ruminants infected by flies of the genus Culicoides (Tabachnick 2004; Sperlova and Zendulkova 2011). There are symptoms in sheep such as high fever, drooling, mucopurulent nasal discharge, ulceration in the oral mucosa and necrosis. In addition, high morbidity and low mortality are observed in animals. Cyanosis occurs in the mouth lesions of heavily infected animals and the appearance of a dark blue tongue is the characteristic finding of the disease. The disease is on the list of notifiable diseases in the world (Sperlova and Zendulkova 2011; Maclachlan 2011).

Acute phase response (APR), which is a reaction of the organism against tissue damage, inflammation, infection, causes the production and release of certain proteins known as acute phase proteins (APP) produced in hepatocytes and peripheral tissues (Iliev and Georgieva, 2018). While the blood levels of some APPs increase, others decrease. While haptoglobin and serum amyloid A (SAA) are very important in ruminants, ceruloplasmin is a moderately important APP. The concentrations of these APPs in circulation are generally related to severity of the disorder and extent of the tissue damage.

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Therefore, measurement of serum haptoglobin, SAA and ceruloplasmin concentrations in ruminants can be used for diagnostic and prognosis as well as evaluation of APR (Tothova et al., 2014; Kuru et al. 2015).

Free radicals produced by the mitochondria during normal oxygen use of the organism cause oxidative damage by creating changes in the structure of lipids, proteins and nucleic acids (Karabulut and Gülay, 2016). The system that works in order to prevent the damage caused by the free radicals is defined as antioxidant system and this system acts by preventing the radical production and/or eliminating the harmful effects of the formed radicals (Süleyman et al. 2018). Normally, while there is a balance between the oxidant and antioxidants in the organism; stress, chronic diseases and infections in the organism stimulate the immune system, as a result, tissue damage occurs due to the increase in the amount of free radicals (Atmaca et al., 2015; Karabulut and Gülay, 2016).

The studies have reported that the total oxidant/antioxidant capacity (TOC, TAC) and oxidative stress index (OSI) will be able to change in cases of the local and/or systemic inflammation or infection and can be used as non-invasive marker (Celi and Gabai, 2015; Aydoğdu et al., 2018).

In this study, it was aimed to determine the diagnostic importance of haptoglobin, SAA, ceruloplasmin, albumin, TAC, TOC and OSI levels in sheep diagnosed with the bluetongue virus disease.

MATERIAL and METHOD

The study was carried out in 25 bluetongue virus-infected and 10 healthy sheep raised in Kars and its districts. Blood samples taken from the Vena jugularis of the animals were taken into tubes with and without anticoagulant. Samples were stored at -20 °C until analysis. Both groups used in the study consisted of animals not vaccinated against bluetongue virus disease.

RNA extraction and reverse transcriptase-polymerase chain reaction (RT-PCR)

RNA was extracted from the samples using a High Pure Viral RNA Kit (Roche, Mannheim, Germany) and complementary DNA (cDNA) synthesis was performed using a RevertAid first-strand cDNA synthesis kit (Thermo Fisher Scientific, USA), according to the manufacturers'instructions. RT-PCR was performed using the method and primer (PP-1) described by Nikolakaki et al. (2005). The formation of PCR products of the expected size (822 bp) was analysed by DNA gel electrophoresis.

Biochemical analysis

The TOC and TAC levels have been determined by using a commercial test kit (Rel Assay Diagnostics, Turkey). The OSI has been calculated by using the formula [TOC (μmol H2O2 equivalents/L)/10 x TAC (mmol Trolox equivalents/L)] (Karababa et al., 2013). The serum haptoglobin concentration has been determined with determining the hemoglobin binding capacity described by Skinner et al. (1991). Ceruloplasmin has been determined by the colorimetric method based on the p-phenylenediamine oxidase activity described by Colombo and Richterich (1964). SAA has been determined by ELISA test kit (Tridelta phase range, Ireland), and albumin has been determined in accordance with the procedure with a commercial test kit (Biolabo, France).

Statistical analysis

SPSS for Windows 20.0. was used for the statistical analyses. The distribution of the data obtained from the groups were shown as normal distribution according to the Kolmogorov-Smirnov test. Therefore, Student-t test was used to compare sheep infected with bluetongue virus disease and control group.

RESULTS

RT-PCR: The amplification of bluetongue virus disease specific 822 bp fragment from RNA of samples and positive control were described as positive reaction (Figure 1).

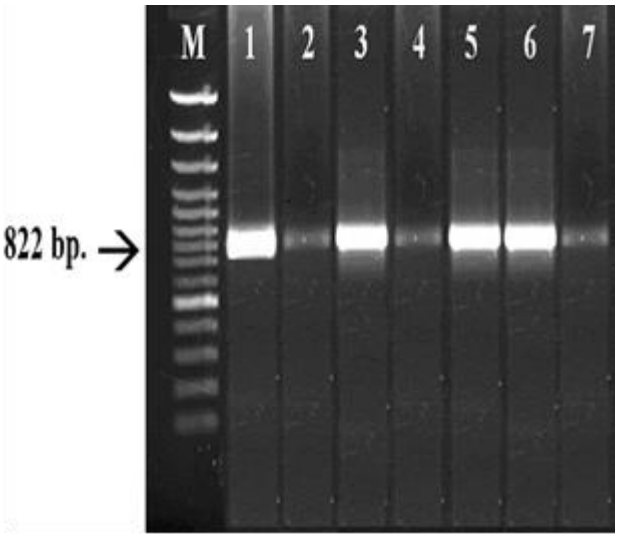

Figure 1. The result of bluetongue virus disease RT-PCR in serum samples. Line M: 100 bp DNA ladder, Line 1: Positive control (822 bp), Lines 2-7: Positive samples.

When comparing sheep infected with bluetongue virus disease to healthy sheep, it has been determined that the concentration of SAA, TOC and OSI (P<0.001) increased, albumin (P<0.05) and TAC value (P<0.01) decreased compared to the control group, in addition, haptoglobin and ceruloplasmin levels increased, however, has been found to be statistically insignificant (Table 1).

Table 1. Some acute phase proteins, TAC&TOC levels in healthy sheep and infected with bluetongue virus disease. Data are presented as mean±standard error (X±SEM).

Parameters	Control (n=10)	Infected (n=25)	P
Haptoglobin (mg/L)	161±17	177±9	NS
SAA (mg/L)	21.87±1.77	67.99±3.57	P<0.001
Ceruloplasmin (mg/dL)	12.74±0.75	14.12±0.61	NS
Albumin (g/dL)	3.39±0.13	3.04±0.07	P<0.05
TAC (mmol Trolox Equiv/L)	0.92±0.06	0.65±0.04	P<0.01
TOC (µmol H_2O_2 Equiv/L)	21.83±2.56	36.24±1.71	P<0.001
OSI (Arbitrary Unit)	2.42±0.28	6.02±0.44	P<0.001

DISCUSSION

In the event of chronic disease, stress or infection in the organism, the amount of free radicals increases by the cells making phagocytosis in the immune system and tissue damage occurs (Aydoğdu et al., 2018). Although there are many methods for determining the oxidative stress, which plays a role in the pathogenesis of many diseases and inflammatory conditions, these methods are complex and expensive methods that require long time and effort and allow the measurement of oxidant/antioxidant molecules one by one to evaluate only for the molecule being measured. For this reason, it has been reported that the measurement of TAC and TOC is easier than the measurement of individual oxidant/antioxidant in order to determine the oxidant/antioxidant balance. The OSI, which is defined as the ratio of TOC level to TAC level, is an indicator of the oxidative stress level (Erel 2004; Erel 2005). It has been stated that the oxidative stress develops in the viral, bacterial and parasitic diseases such as foot-and-mouth disease (Deveci et al., 2018), brucella (Merhan et al., 2017a), hypodermosis (Merhan et al., 2017b) and cryptosporidiosis (Çenesiz et al., 2017) in cattle and pox virus infected sheep (Bozukluhan et al., 2018), the antioxidant level decreases while the oxidant level increases. It has been stated that the cell and tissue damages occur with increasing the amount of free radicals in the organs and tissues (Küçük 2021).

In our study we conducted that TOC and OSI (P<0.001) values increased and TAC (P<0.01) values decreased in sheep infected with the bluetongue virus disease. Therefore, it is seen that the findings we have obtained are compatible with the above-mentioned studies.

While the serum level of positive APPs increased in the liver as a result of the inflammation, tissue damage and infection; it is stated that the negative APPs decrease. Haptoglobin has many functions such as forming stable complexes with the free hemoglobin and thus creating a bacteriostatic effect by preventing iron loss, as well as regulating the lipid metabolism and stimulating the immune system as an immunomodulator (Petersen et al., 2004). Haptoglobin, which is a positive APP, has been reported to increase in bacterial (Bozukluhan et al., 2016), viral (Merhan et al., 2017c) and parasitic (Bozukluhan et al., 2017; Merhan et al., 2017b) diseases. It has been reported that SAA, another positive APP, will be able to be used in determining the severity and prevalence of inflammatory events, prognosis and evaluating the success of the treatment applied (Tothova et al., 2014). The SAA, which has functions such as transporting cholesterol to hepatocytes, preventing oxidative destruction of neutrophils, stimulating calcium release from monocytes; has been reported to increase in bacterial (Kaya et al., 2016), viral (Merhan et al., 2017c) and parasitic infections (Merhan et al., 2017b). In a study conducted in sheep infected with the bluetongue (Aytekin et al., 2015), it has been reported that the sialic acid levels increased, while the albumin and ceruloplasmin levels decreased. In another study conducted in sheep infected with the bluetongue, it has been reported that the haptoglobin, SAA and ceruloplasmin levels increased, while the albumin levels decreased (Sanchez-Cordon et al., 2013). In this study, it has been determined that while the SAA (P<0.001) and haptoglobin levels, which are major APPs, increased in ruminants, the albumin (P<0.05) levels decreased, and it is thought that this situation will be able to be related to the tissue destruction.

The ceruloplasmin, an α-2 globulin, has functions such as copper transport, oxidation of the toxic iron to the non-toxic iron, and antioxidant effect. Copper increases the immune function by affecting on various enzyme levels that mediate the antioxidant system. The ceruloplasmin mediates for the copper transport to lysyl oxidase and copper-zinc superoxide dismutase enzymes by involving in the tissue repair and plays a role in the antioxidant system. It also protects the cells against the oxidative damage. If the serum ceruloplasmin level decreases, the phagocytosis and antimicrobial activity also decrease. Therefore, the need for this enzyme increases in the inflammatory conditions (Cerone et al., 2000). Studies have reported that its concentration increases in bacterial (Bozukluhan et al., 2016), viral (Merhan et al., 2016) and parasitic diseases (Nisbet et al., 2008; Bozukluhan et al., 2020). In the study, although the ceruloplasmin level increased in sheep infected with the bluetongue, it was found to be statistically insignificant. The reason for the increase in the ceruloplasmin concentration is thought to be due to the increase in the antimicrobial and phagocytolytic effects of the cells in the defense system.

CONCLUSION

In conclusion, sheep infected with bluetongue virus disease was detected to cause important changes in the oxidative-antioxidative capacity and APP levels in cattle. It has been concluded that the SAA will be able to be used as an inflammation marker in sheep infected with the bluetongue.

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Ethical approval:

Studies were performed by Erciyes University Animal Testing Local Ethics Council (ERU-2013/102).

Conflict of interest: No potential conflict of interest was reported by the author.

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