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Research Article

Evaluation of Paraoxonase Activity, Total Sialic Acid and Oxidative Stress in Sheep with Ecthyma Contagiosa [1]

Hacı Ahmet DEVECİ [1] Abdulsamed KÜKÜRT [2] Erdoğan UZLU [3]
İbrahim SÖZDUTMAZ [4] Oğuz MERHAN [2] Sinan AKTAŞ [5]
Merve ALPAY [6] İnan KAYA [7] Mahmut KARAPEHLİVAN [8]

[1] *A part of this study has been presented in Cancer Genome: From Structure to Function Congress, 11-14 May, 2014, Malaga, Spain*

[1] Gaziantep Üniversitesi, Islahiye Meslek Yüksekokulu, TR-27800 Islahiye, Gaziantep - TÜRKİYE
[2] Kafkas Üniversitesi, Veteriner Fakültesi, Biyokimya Anabilim Dalı, TR-36100 Kars - TÜRKİYE
[3] Kafkas Üniversitesi, Veteriner Fakültesi, İç Hastalıkları Anabilim Dalı, TR-36100 Kars - TÜRKİYE
[4] Erciyes Üniversitesi, Veteriner Fakültesi, Viroloji Anabilim Dalı, TR-38000 Kayseri - TÜRKİYE
[5] Ataturk Üniversitesi, Veteriner Fakültesi, İç Hastalıkları Anabilim Dalı, TR-25100 Erzurum - TÜRKİYE
[6] Düzce Üniversitesi, Tıp Fakültesi, Tıbbi Biyokimya Anabilim Dalı, TR-81620 Düzce - TÜRKİYE
[7] Kafkas Üniversitesi, Fen Edebiyat Fakültesi, Moleküler Biyoloji ve Genetik Bölümü, TR-36100 Kars - TÜRKİYE
[8] Kafkas Üniversitesi, Tıp Fakültesi, Tıbbi Biyokimya Anabilim Dalı, TR-36100 Kars - TÜRKİYE

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Abstract

Zoonoses are defined by the world health organization as diseases and infections which are transmitted naturally between vertebrate animals and human. Undestanding the zoonotic risk posed by pox viruses in companion animals is important for both human and animal health. Contagious ecthyma is highly contagious, zoonotic, viral skin disease that affects sheep, goats and some other domesticated and wild animals. In this present study was detected and evaluated levels of plasma paraoxonase activity (PON1), high-density lipoprotein (HDL), total sialic acid (TSA), malondialdehyde (MDA), nitric oxide (NO) and total blood glutathione (GSH) concentrations in healthy sheep and natural infected sheeps with ecthyma. In healthy sheep, laboratory results were determined as PON1 218.54±17.93 U/L, TSA 59.89±5.59 mg/dL, HDL 48.4±4.88 mg/dL, MDA 8.58±0.80 µmol/L, NO 7.78±1.02 µmol/L and GSH 21.11±3.70 mg/dL. These values were found 174.92±18.68 U/L, 70.1±6.56 mg/dL, 37.9±6.47 mg/dL, 11.26±1.06 µmol/L, 12.44±1.90 µmol/L, 7.79±0.90 mg/dL respectively in sheeps wich are infected by ecthyma. As a result, it was concluded that there is oxidative stress due to imbalance between pro-oxidant and antioxidant molecules in sheep which are infected by ecthyma, and this imbalance is shaped by increasing oxidant levels.

Keywords: *Ecthyma, Paraoxonase activity, Total sialic acid, Oxidative stress, Sheep*

Ecthyma Contagiosa'lı Koyunlarda Paraoksonaz Aktivitesi, Total Sialik Asit ve Oksidatif Stresin Değerlendirilmesi

Özet

Zoonozlar, dünya sağlık organizasyonu tarafından omurgalı hayvanlar ve insanlar arasında doğal olarak iletilen hastalıklar ve enfeksiyonlar olarak tanımlanmaktadır. Pox virüslü hayvanların oluşturduğu zoonotik riskin saptaması ve bertaraf edilmesi hem insan hem de hayvan sağlığı için önemlidir. Bulaşıcı ektima koyun, keçi, diğer bazı evcil ve vahşi hayvanları etkileyen bulaşıcı, zoonotik, viral cilt hastalığıdır. Sunulan bu çalışmada sağlıklı ve ektima ile doğal enfekte koyunlarda plazma paraoksonaz aktivitesi (PON1), yüksek dansiteli lipoprotein (HDL), toplam sialik asit (TSA), malondialdehit (MDA), nitrik oksit (NO) ve toplam kan glutatyonu seviyeleri tespit edildi ve sonuçları değerlendirildi. Sağlıklı koyunlarda PON1 218.54±17.93 U/L, TSA 59.89±5.59 mg/dL, HDL 48.4±4.88 mg/dL, MDA 8.58±0.80 µmol/L, NO 7.78±1.02 µmol/L ve GSH 21.11±3.70 mg/dL olarak belirlendi. Ektima ile enfekte koyunlarda ise bu değerler sırasıyla 174.92±18.68 U/L, 70.1±6.56 mg/dL, 37.9±6.47 mg/dL, 11.26±1.06 µmol/L, 12.44±1.90 µmol/L, 7.79±0.90 mg/dL tespit edildi. Sonuç olarak, ektima ile enfekte koyunlarda, pro-oksidan ve antioksidan moleküller arasındaki dengesizliğe bağlı oksidatif stres oluştuğu, bu dengenin artan oksidan seviyelerine bağlı olduğu saptandı.

Anahtar sözcükler: *Ektima, Paraoksonaz aktivitesi, Total sialik asit, Oksidatif stres, Koyun*

İletişim (Correspondence)
+90 532 2757135
euzlu@hotmail.com

INTRODUCTION

Ecthyma contagiosum *(Contagious ecthyma, Orf)* mainly occures in sheep and goats. The disease is caused by an epitheliotropic virus *(Parapoxvirus -Orfvirus-* of the family Poxviridae), and is common disease in Türkiye, it can affects many small ruminant spicies between spring and autumn. Orf has been reported in people who handled infected animals or there tissues. Contagious ecthyma results from infection by the orf virus, a member of the genus parapox virus in the sub family chordopox viridae. Ecthyma contagiosum mainly occures in sheep and goats. Orf has been reported ın people who handled infected animals or there tissues. It is mandatory to report this zoonotic disease that causes significant economic loss in animal husbandry and can be transmitted to humans from animals [1-3]. Ecthyma contagiosum disease is known as orf, contagious ecthyma, contagious pustular stomatitis, infectious labial dermatitis, infectious pustular dermatitis, sore mouth, and scabby mouth [4].

Orf virus can be transmitted by direct contact with sick animals or indirectly from materials such as dried skin that fall on the grass during grazing. Infection can also be transmitted as a result of the administration of salt blocks or licking stones, which have contacted with infected wild goats. It has also known that the rate of spread of the disease and the size of the lesions are related to the labor exposure which causes injuries on the mouths of animals during grazing [3]. It has been reported by different researchers that the disease affects all age groups equally, without racial and gender discrimination between animals [5-7].

In ecthyma contagiosum, lesions are mainly seen around the lips and around. In addition, typical lesions can be seen in mouth, nose, oral mucosa, tongue, gingiva and palate. Similar lesions are observed in the lambs as small erythematous and ulcerated papules on the gums, tongue and palate [2,3,5,8,9]. Skin lesions of the disease are frequently seen in the head, face, ears, neck, chest, legs and inguinal region [10]. The lesions can usually be cauliflower-like papillomatosis, proliferative, granulomatous, dry crusted, embossed and erosive [5]. The average incubation period in ecthyma contagiosum disease is 2-3 weeks [11,12]. Diagnosis of the disease is easily possible with clinical findings, characteristic skin lesions and histopathological examination findings of these lesions as well as biopsy of the infected skin and visualization of the agent itself in electron microscopy or advanced laboratory techniques like PCR [11].

Reactive oxygen species or free radicals are released from dendritic cells, neutrophils and macrophages in response to an inflammatory agents. These materials are highly reactive because they contain unpaired electron or non-static bonds. Free radicals and antioxidant mechanisms are in balance in normal physiological condition. In order to limit an effective immune response and tissue damage with the living organism, it is vital that these substances are in balance [13,14]. The increase in the level of reactive oxygen species in cells leads to oxidative damage in protein, lipid and DNA. In this case, loss of enzyme activity, inhibition of protein synthesis and DNA damage occur, resulting in cell death [14].

The aim of this study is to determine the levels of plasma paraoxonase activity (PON1), total sialic acid (TSA) and some oxidative stress markers in Akkaraman sheep that are determined to be infected by ecthyma, a natural and important zoonotic viral disease, and to reveal possible changes of these parameters in ecthyma contagiosum disease.

MATERIAL and METHODS

Experimental Animals

Twenty Akkaraman sheep were used in the study at the age of 1 year in the flock which was examined due to the suspicion of ecthyma disease in the Erzurum (Türkiye) region. According to the clinical and laboratory studies animals divided into two groups: Those who were infected with the ecthyma virus (n=10) and those who were healthy (n=10). Blood samples were taken from the vena jugularis of the sheep for laboratory diagnosis of the disease and were centrifuged at 3.000 rpm for 10 min to separate the plasmas. The obtained plasmas were kept at -25ºC until biochemical analyses.

Biochemical Assays

PON1 activity measurement was performed according to the methods of Eckerson [15] and Gülcü and Gürsu [16]. PON1 activity was determined by spectrophotometric measurement of the absorbance of the colored compound, 4-nitrophenol, from the enzyme hydrolysis product paraoxone (Sigma) at 25°C and 412 nm. For PON1 activity, enzyme in 1 mL of serum was identified as the enzyme activity unit that converts 1 nmol of paraoxon to 4-nitrophenone in 1 min and the results are given as U/L. TSA values were measured using a spectrophotometer (PowerWave XS, BioTek®, USA) according to the colorimetric method of Sydow [17] and the results were expressed in mg/dL. High-density lipoprotein (HDL) was studied in the autoanalyzer using the Biotrol trademark kit and the results were shown in mg/dL. Plasma nitric oxide (NO) levels were determined by the method reported by Miranda et al.[18]. In this method, nitrate is converted to nitrite with vanadium (III) chloride. Reaction of N- (1-Naphthyl) ethylenediaminedihydrochloride with nitrite sulfanil-amine in acidic medium gave the resultant complex diazonium compound. The resulting colored complex was measured at 540 nm. After nitrate and nitrite levels were determined separately, the sum of the two was determined as NO amount. The level of malondialdehyde (MDA) was determined according to the method reported by

Yoshioka et al.[19]. The MDA formed in this method forms a pink complex with thiobarbituric acid (TBA) and the absorbance of this solution is measured spectrophotometrically at 535 nm (PowerWawe XS, Biotek®, Instruments, USA) to determine the degree of lipid peroxidation. The MDA levels obtained were calculated as µmol/l. The level of glutathione (GSH) was determined from whole blood according to the method reported by Beutler [20].

DNA Extraction

Scabs were collected from the lip lesions on affected animals and were used for DNA extraction. DNA was extracted from scabs and by using QIAamp DNA Mini kit™ (Qiagen). Scabs were separately placed in a 2 mL screw capped tubes including glass particles with lysis buffer and proteinase K supplied with the kit and homogenised using Magna Lyser™ instrument (Roche). Further extraction protocol was applied according to the manufacturer's instructions, eluted with 100 µL elution buffer and stored at -20°C.

Polymerase Chain Reaction (PCR)

PCR reaction was done with 045-F (5'-CCT ACT TCT CGG AGT TCA GC-3') and 045-R (5'-GCA GCA CTT CTC CTC GTA G-3') primers encoding 392 bp of VLTF-1 gene as described before [19]. PCR was conducted in a 50 µL reaction mixture comprising 25 µL 2X PCR Master Mix (Promega) containing: 50 units/mL of Taq DNA polymerase supplied in a proprietary reaction buffer (pH 8.5), 400 µM dATP, 400 µM dGTP, 400 µM dCTP, 400 µM dTTP, 3 mM $MgCl_2$; 20 pmol each forward and reverse primer, 2 µL template DNA and remained amount of PCR grade water. The reaction was carried out in conditions 40 cycles of denaturation at 95°C for 10 s, annealing at 47°C for 10 s, extension at 74°C for 10 s followed by final extension at 78°C for 10 min.

PCR products were electrophoresed on 2% agarose gel (Sigma®) in Tris-acetate EDTA (TAE) buffer and stained with ethidium bromide (1 µg/mL). Gel was analyzed with a gel documentation system (GeneLine, Spectronics Corp. NY, USA) [21].

Reference control of *Orfvirus* which was previously identified with PCR from field epizooties was kindly provided by Veterinarian Ömer Faruk KÜÇÜKKALEM (Virology Department, Veterinary Control and Research Institute, Erzurum, Turkey). Negative control samples were collected from slaughtered sheep in abattoir without any clinical history and Orf symptoms.

Statistics

The SPSS program was used to evaluate the data obtained from the study. First, the Kolmogorov-Smirnov test was performed and the normal distribution of the groups was assessed and the Student's t test was used to compare these groups with normal distribution. The results are expressed as "mean value (X)±standard deviation (SD)".

RESULTS

As a result of clinical examination of the animals evaluated in the diagnosis of this disease, which is mandatory to report, clinical symptoms were observed as fatigue and hypersalivation as well as typical lesions in the mouth, lips, gums, tongue and nose *(Fig. 1)*.

PON1 activity, TSA, HDL, MDA, NO and GSH levels were measured in the blood samples taken from the animals and PCR evaluation was performed to confirm the diagnosis. In biochemical evaluations; plasma PON1 activity, HDL, and GSH levels obtained from the animals in the contagiosum disease group were statistically significantly low (P<0.001)

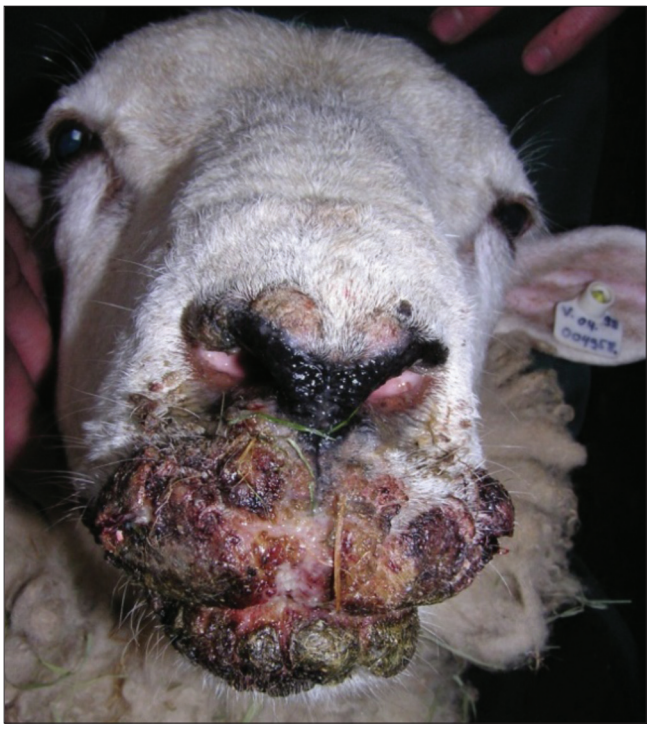

Fig 1. Clinically view of the ecthyma contagiosa in a Akkaraman sheep

Table 1. *Plasma PON1 activity, HDL, TSA, NO, MDA, and whole blood GSH levels in healthy and sheeps with ecthyma*

Parameters	Healty Group (n=10)	Ecthyma Contagiosum (n=10)
TSA (mg/dL)	59.89±5.59[a]	70.1±6.56[b]
PON 1 (U/L)	218.54±17.93[a]	174.92±18.68[b]
HDL (mg/dL)	48.4±4.88[a]	37.9±6.47[b]
MDA (µmol/L)	8.58±0.80[a]	11.26±1.06[b]
NO (µmol/L)	7.78±1.02[a]	12.44±1.90[b]
GSH (mg/dL)	21.11±3.70[a]	7.79±0.90[b]

$X^{a,b}$: The difference between the averages with different letters in the same line is important (P<0.001)

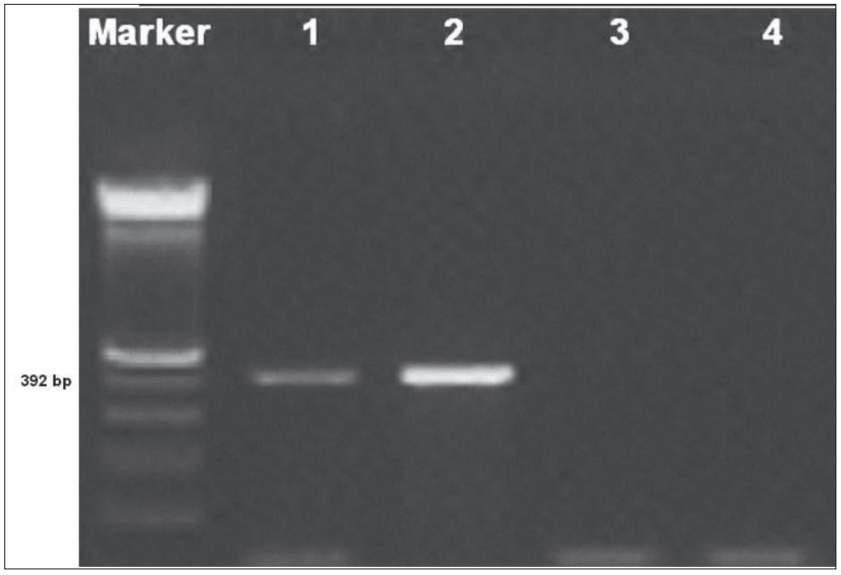

Fig 2. Agarose gel electrophoresis of PCR products: Marker 100 bp DNA ladder (Gene Ruler™, Fermentas), lane 1: Field sample collected in this study, lane 2: Positive control, lane 3: Negative control, lane 4: Water

compared to the healthy animal group. On the other hand, on the ecthyma contagiosum group TSA, MDA and NO levels were found to be statistically significantly ($P<0.001$) higher than the control group *(Table 1)*.

All of the clinical samples were detected positive with PCR *(Fig. 2)* in ecthyma group. Targeted 392 bp of PCR product was visualised from the DNA extracts of positive control sample and scab materials collected in this study. Targeted specific PCR amplicons were not detected from negative control samples and water.

DISCUSSION

It has been reported that in the case of ecthyma contagiosum disease, typical lesions such as papules, pustules, nodules have been observed following clinical manifestations of weakness, anorexia, hypersalivation, tongue and swelling and redness in the gums [2,11,12]. Typical skin lesions in the clinical examination of the animals used in our study were observed in the group of patient sheep, suggesting similar symptoms in addition to the usual symptoms.

NO is a biological mediator synthesized from L-arginine by nitric oxide synthase catalysis and cytotoxic to agents such as bacteria, fungi, protozoa and viruses [3,14,22]. Free radicals or reactive oxygen species are released from neutrophils and macrophages during inflammatory conditions [13]. These species are toxic to biomembranes. Unless removed by free radical cleansing enzymes such as glutathione peroxidase (GSH-px), they cause peroxidation of lipids. In such cases, antioxidants act to purify free radicals by converting them into less harmful molecules [23]. It has been reported that the paratoxyl-orf virus influences neutrophil, basophil, and mast cells from the inflammatory cells, in spite of the early response of multiplying neutrophils in the early period of viral replication and acts against disease, the number of cutaneous mast cells does not alter [24,25]. In our study, the level of NO obtained from sick animals was higher than that of control group, and the level of GSH obtained from whole blood was found to be low. This increase in NO levels in ecthyma infection is thought to be caused by release of too much free radicals due to the increase in neutrophils that became active because of the disease which are reported by researchers [3]. The decrease in the level of GSH that we have found is in line with the view that researchers previously reported that free radicals, which are found in inflammatory conditions, strongly consume antioxidants.

The increase in the level of reactive oxygen species in the cells results in oxidative damage to the structure of proteins, lipids and DNA [14]. MDA, the final product of lipid peroxidation and the most important indicator, is the most important molecule effective in cellular degeneration caused by free radicals [26]. In the present study, it was determined that the MDA concentration obtained from the group of sheeps with ecthyma was significantly higher than that of the other group and lower GSH and HDL levels. It has been determined that high MDA and low GSH and HDL levels from the group of sheeps with ecthyma were found to be an important indicator of lipid peroxidation in ruminants, and are particularly compatible with studies conducted by researchers in poxvirus, some other viral, bacterial and parasitic agents [23,26-30].

Sialic acid, an acetylated derivative of neuraminic acid, is an important cell surface component found in biological membranes of bacteria and animals. Many investigators have reported that TSA concentration increases in cases of severe inflammatory, cellular degeneration or proliferation situations [30-32]. In this study, it was determined that the TSA concentration obtained from parapox-orf virus-infected sheep was statistically significantly higher than healthy sheep. The high level of TSA in infected sheep is thought to be due to severe cellular degeneration and proliferation caused by inflammation in these animals.

PON1 is an antioxidant enzyme found in liver, kidney, intestine and HDL on serum and its activity can change as part of the inflammatory response [32,33]. In this presented study, a decrease in both HDL and PON1 activity was detected in the sheeps with ecthyma. The cause of this reduction may be the lipid peroxidation, which the severity is evidenced by other markers. This has been confirmed

in agreement with investigators who report that oxidized lipids inhibit PON1 activity in elevated lipid peroxidation [33-35].

In conclusion, low PON1 activity, HDL, GSH levels and high MDA and NO levels, which were obtained from the sheeps were infected by ecthyma. That indicate the disease caused significant oxidative stress in sheep. Along with that, it was concluded that the high TSA level determined in the group consisting of patient sheeps was an important indicator of oxidative stress-induced cell and tissue damage in the disease.

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