Acta Veterinaria-Beograd 2026, 76 (1), 66-87
UDK: 636.32/.38.09:616.24-022.7
636.32/.38.09:616-008.9:[577.334:546.21
DOI: 10.2478/acve-2026-0005

Research article

OXIDATIVE STRESS AND ADENOSINE DEAMINASE ACTIVITY IN SHEEP WITH PULMONARY ADENOCARCINOMA

Şemistan KIZILTEPE[1*], Emin KARAKURT[2], Gizem ESER[3], Nüvit COŞKUN[4], Selda GÜNEŞ[5], Çağlar OKULMUŞ[6], Oğuz MERHAN[7]

[1]Igdır Unıversıty Tuzluca Vocational School, Department of Medical Services and Techniques, Iğdır, Türkiye; [2]Kafkas University, Faculty of Veterinary Medicine, Department of Pathology, Kars, Türkiye; [3]Muğla Sıtkı Koçman University, Faculty of Milas Veterinary Medicine, Department of Veterinary Pathology, Milas/Muğla, Türkiye; [4]Kafkas University, Faculty of Veterinary Medicine, Department of Virology, Kars, Türkiye; [5]Kafkas University, Faculty of Veterinary Medicine, Department of Pathology, Kars, Türkiye; [6]Department of Biochemistry, Dokuz Eylül University, Faculty of Veterinary Medicine, Buca/İzmir, Türkiye; [7]Kafkas University, Faculty of Veterinary Medicine, Department of Biochemistry, Kars, Türkiye.

(Received 06 November 2025, Accepted 06 March 2026)

Ovine pulmonary adenocarcinoma (OPA) is an infectious and neoplastic disease etiologically linked to the Jaagsiekte sheep retrovirus (JSRV), characterised by tumour lesions of the lung. Because of the economic losses it induces, OPA is of great importance for flock health. In this study, oxidative stress markers and adenosine deaminase (ADA) activity were quantified in lung tissue from sheep, both healthy and those naturally afflicted with OPA. Compared to healthy sheep, malondialdehyde (MDA), nitric oxide (NO), ceruloplasmin (CP) and ADA concentrations/activities were significantly increased ($P<0.05$ and $P<0.001$) in fresh lung tissues from JSRV–infected sheep, while reduced glutathione (GSH) levels were significantly decreased ($P<0.05$). In conclusion, pronounced oxidative stress and increased ADA enzyme activity were detected in the JSRV–infected sheep. These findings suggest that ADA activity could serve as a biomarker for disease diagnosis.

Keywords: Pulmonary Adenocarcinoma, Oxidative Stress, Adenosine Deaminase, Sheep.

INTRODUCTION

Ovine pulmonary adenocarcinoma (OPA) is a transmissible neoplastic condition characterized by tumor lesions in the lungs, induced by the Jaagsiekte sheep retrovirus (JSRV) [1,2]. The disease has been identified worldwide in several sheep and goat breeds and has a profound impact on flocks, causing significant economic losses. The causative agent belongs to the *Retroviridae* family, the *Orthoretrovirinae* subfamily,

*Corresponding author: e-mail: semistan.kiziltepe@igdir.edu.tr

and the *Betaretrovirus* genus. OPA originates from oncogenically transformed type II pneumocytes and Clara cells, which are situated in the distal airways [3,4]. Although JSRV can infect various cell types, pathological changes are primarily confined to the lungs due to its transcriptional activity in differentiated airway epithelial cells [3]. Transmission primarily occurs via aerosol. However, oral transmission has also been observed in lambs. The first clinical sign of the disease is progressive respiratory insufficiency [5,6]. Weight loss occurs despite having normal appetite and feed intake during the same period, and is considered a significant clinical sign [5,7,8].

Viral infections and neoplastic processes disrupt the physiological balance of the host and lead to oxidative stress. Oxidative stress is a condition in which the compensatory antioxidant system cannot control the excessive production of Reactive Oxygen Species (ROS) or Reactive Nitrogen Species (RNS). The excessive production of these reactive species causes DNA damage in cells [9,10]. Oxidative stress–induced DNA damage in cells plays a critical role in transforming normal cells into uncontrollably dividing cancer cells, and it is considered an essential mechanism in the pathogenesis of many types of cancer [11].

At physiological concentrations, ROS act as signaling molecules, but when they exceed homeostatic limits, they damage macromolecules such as DNA, lipids, and proteins. Malondialdehyde (MDA), a product of lipid peroxidation, is a toxic compound that reacts with DNA and proteins, causing oxidative damage in cells. This property makes MDA a common biomarker of cell membrane lipid peroxidation [10]. Nitric oxide (NO) is a free radical synthesized by the nitric oxide synthase (NOS) enzymes and participates in various physiological and pathological processes [12,13]. NO has dual effects in cancer pathogenesis, promoting tumor growth or eliminating tumor cells. One of the primary antioxidants protecting the lungs against these oxidants is Glutathione (GSH). When the balance between oxidants and antioxidants is disrupted, the destructive effects of oxidants may result in metabolic disorders and cellular demise [10].

Ceruloplasmin (CP), a liver–synthesized acute-phase protein, is stimulated by inflammatory mediators. In pathological conditions, CP plays an important role as an antioxidant against oxygen radicals released by immune cells in areas of inflammation. CP also has prognostic significance in patients with lung adenocarcinoma and malignant tumors [14-17]. Adenosine deaminase (ADA) is an essential enzyme in purine metabolism that regulates intracellular and extracellular adenosine concentrations [18]. ADA is found in all body tissues and fluids; in addition to its immunosuppressive effect, it acts as a signal for tissue damage and inflammatory changes.

Preclinical diagnosis of OPA is limited because neither routine serology nor virus isolation and cell culture methods are widely available. Diagnosis relies primarily on clinical signs and postmortem pathological lesions, although it is also possible to use molecular methods such as the reverse transcription polymerase chain reaction (RT–PCR) [2,5,19]. Given these diagnostic limitations and the limited understanding of

OPA pathogenesis, investigating various biochemical markers is of great value. In this study, the concentrations of oxidative stress indicators such as MDA, NO, GSH, and CP, along with ADA activity, were measured in lung tissues from sheep naturally infected with JSRV to determine the involvement of these biochemical parameters in the pathogenesis of the disease and to explore their potential use as biomarkers for diagnosing OPA.

MATERIAL AND METHOD

The study was conducted with the permission numbered KAÜ-HADYEK/2025-102 obtained from the Kafkas University Animal Experiments Local Ethics Committee (KAÜ-HADYEK).

For this study, samples of lung tissue exhibiting a frosted–glass appearance, a macroscopic characteristic of the disease, were collected during postmortem examinations of sheep aged 1–5 years that had been slaughtered at a commercial abattoir. For pathological examination, tissues were preserved in 10% formaldehyde solution, while samples for virological and biochemical examination were kept at –20°C until analysis. Lung tissue samples were obtained from healthy sheep to form a control group for comparison with OPA–infected tissues.

The causative agent was identified by reverse transcriptase polymerase chain reaction (RT–PCR) from RNA extracted from frozen tissue samples.

Biochemical analyses were also performed on homogenates prepared from OPA and healthy tissues. Histopathological examinations were performed on samples in which the disease was detected. ADA activity, NO, glutathione (GSH), MDA, albumin (Alb), and CP levels in tissue homogenates were measured in the biochemistry laboratory.

Biochemical parameters

The concentrations of MDA, NO, reduced GSH, and ceruloplasmin were quantified as described by the methods outlined by Yoshioka et al. [20], Miranda et al. [21], Beutler et al. [22], and Colombo and Richterich [23], respectively. Adenosine deaminase (ADA) activity (Elabscience, China), albumin, and total protein (Biolabo, France) were measured calorimetrically using commercial assay kits (Epoch, Biotek, USA).

Histopathological examinations

Lung tissue samples obtained from sheep suspected of being infected with JSRV underwent fixation in 10% formaldehyde solution. Following standard tissue processing protocols, serial sections 5 micrometres thick were cut from the prepared paraffin blocks. These sections were subsequently stained with Haematoxylin & Eosin (H&E) to reveal histopathological changes. The prepared slides were comprehensively examined using a light microscope by two distinct independent pathologists. Significant

pathological findings were photographed at various magnifications and recorded separately for each case [24].

For the analysis of the inflammation score, areas with high reaction density were primarily selected. For each case, five different fields in the tumoral tissue were evaluated under a 20× objective lens. The numbers of inflammatory cells in the tumor microenvironment were recorded separately for each case, and the mean of these five fields was accepted as the average positive cell count for that JSRV-infected case. The grading was designed as follows: (−) no inflammatory reaction; (+) mild inflammatory reaction, 1–10% inflammatory cell presence; (++) moderate inflammatory reaction, 11–59% inflammatory cell presence; and (+++) severe inflammatory reaction, more than 60% inflammatory cell presence [25].

Tissue homogenization

Approximately 1 g of lung tissue was dissected and minced with scalpel blades. The minced tissue was placed in a polystyrene tube, and 900 microliters of phosphate buffer were added. The mixture was subjected to vigorous vortex mixing for 1 minute and centrifuged at 3,000 x g for 15 minutes. The supernatant was collected and used for nucleic acid extraction.

Nucleic acid extraction

The RNA was extracted using the guanidinium thiocyanate–phenol–chloroform method outlined by Chomczynski and Sacchi [26]. The extracts were stored at – 20°C until further analysis.

Reverse transcription polymerase chain reaction (RT-PCR)

RT-PCR was conducted using the OneStep RT-PCR kit (Hibrigen, Türkiye) in conjunction with a primer pair, previously documented by Mansour et al. [27], and designed to target the envelope gene region (KT279065.1). The primers are shown in Table 1. Reverse transcription was performed at 55ºC for 40 minutes. The PCR cycle included an initial denaturation at 95ºC for 5 minutes, followed by 35 cycles of 95ºC for 1 minute, 58ºC for 1 minute for annealing, and 72ºC for 1 minute for extension. It concluded with a final extension at 72ºC for 10 minutes. The anticipated size of the amplicon was 398 base pairs. A previously sequenced JSRV sample was used as a positive control (PC) and distilled water was used as a negative control (NC) to assess the RT-PCR test. Amplicons were visualized using a transilluminator after running them on a 1% agarose gel stained with Safe–Red (Safe ViewTM Cat No: G108-R, Canada).

Table 1. Sequence of the primer pairs targeting envelope coding gene

	Sequence	Expected Product Size
Forward	CCGGAAAGAGATCGTACCGT	398 base pairs
Reverse	TAAGGAACACAAGCTCGGGG	

Statistical analyses

Study data were statistically analyzed using the SPSS (Statistical Package for the Social Sciences) software package (version 27.0, SPSS Inc., Chicago, IL, USA). The Shapiro-Wilk test was used to assess whether the groups followed a normal distribution. Comparisons of biochemical parameters and cell inflammatory cell scores between groups were performed using the Mann–Whitney U test. A p value < 0.05 was considered statistically significant.

RESULTS

Macroscopic findings

In the lungs of sheep with classical OPA, highly irregular and firm white–grey colored temporal foci localized in different lobes were observed. It was noted that these lungs did not collapse well. It was determined that classical–type OPA lesions were much more prominent in the cranial, medial, and caudal lobes. In addition, it was noted that these neoplastic areas, which appeared nodular or diffuse, were much more severe in the cranial lobes (predominantly in the cranio–ventral regions). The pleura covering these neoplastic structures exhibited a smooth, glossy, and translucent appearance, whereas the cut surfaces were greyish-white with a frosted-glass texture and showed outward protrusion. In some cases, a significant increase in lung weight and volume was recorded, parallel to the size of the tumor lesions. When sections were excised from the tumor foci, their surfaces were seen to be quite moist.

In sheep with atypical OPA, the lesions were primarily localized in the diaphragmatic lobes of the lungs. These tumor lesions, which varied considerably in size, were well demarcated from the surrounding normal tissue. Compared to classical OPA, the cut surfaces of atypical OPA were remarkably dry. Tumor foci exhibiting solitary or multifocal growths had a firm and solid structure (Figure 1 and 2).

No metastatic foci were detected in regional lymph nodes or distant tissues in either classical or atypical OPA cases.

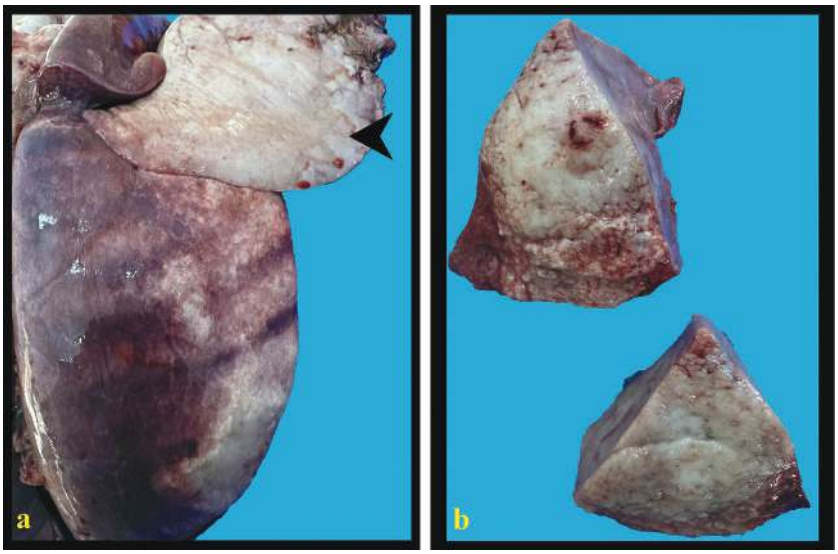

Figure 1. Classical OPA, macroscopic appearance **a:** tumor areas in the cranial lobes (arrowheads) and **b:** cross–sectional surfaces of these cancer foci

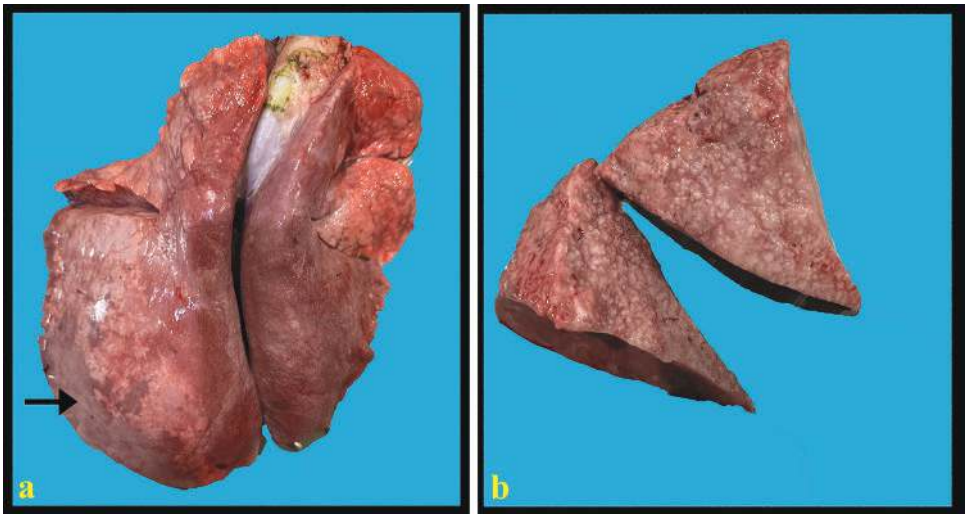

Figure 2. Atypical form of OPA, macroscopic appearance, **a:** greyish–white tumor foci in the diaphragmatic lobes (arrow) and **b:** cross–sectional views of these tumor areas

Histopathological findings

Information regarding the developmental form, tumor proliferation pattern, secondary infection, inflammation score, and lymph node and distant tissue metastasis status of all OPA and control cases is presented in detail in Table 2. It was determined that the

lung tissues of the control group preserved their normal histological structure and did not exhibit any inflammatory reaction.

Table 2. OPA developmental forms, tumour cell proliferation patterns, secondary infections, inflammation scores and metastasis status

Case No.	OPA Form	Proliferation Pattern	Secondary Infection	Inflamattion Score	Lymph Node and Distant Tissue Metastasis
1	Atypical	Papillary and Acinar	Interstitial Pneumonia	++	-
2	Classical	Papillary and Acinar	Catarrhal Purulent Bronchopneumonia	+++	-
3	Atypical	Papillary	Interstitial Pneumonia	+	-
4	Atypical	Papillary	Catarrhal Purulent Bronchopneumonia	+++	-
5	Atypical	Papillary and Acinar	Catarrhal Purulent Bronchopneumonia	++	-
6	Atypical	Papillary	Bronchointerstitial Pneumonia	++	-
7	Classical	Papillary and Acinar	Fibrinous Bronchopneumonia	+++	-
8	Classical	Papillary	Catarrhal Purulent Bronchopneumonia	++	-
9	Classical	Papillary	-	-	-
10	Classical	Lepidic	-	-	-
11	Control	-	-	-	-
12	Control	-	-	-	-
13	Control	-	-	-	-
14	Control	-	-	-	-
15	Control	-	-	-	-
16	Control	-	-	-	-
17	Control	-	-	-	-
18	Control	-	-	-	-
19	Control	-	-	-	-
20	Control	-	-	-	-

In both the classic (n = 5) and atypical (n = 5) forms of OPA, neoplastic cells were observed to be of cubic or columnar type (Figure 3).

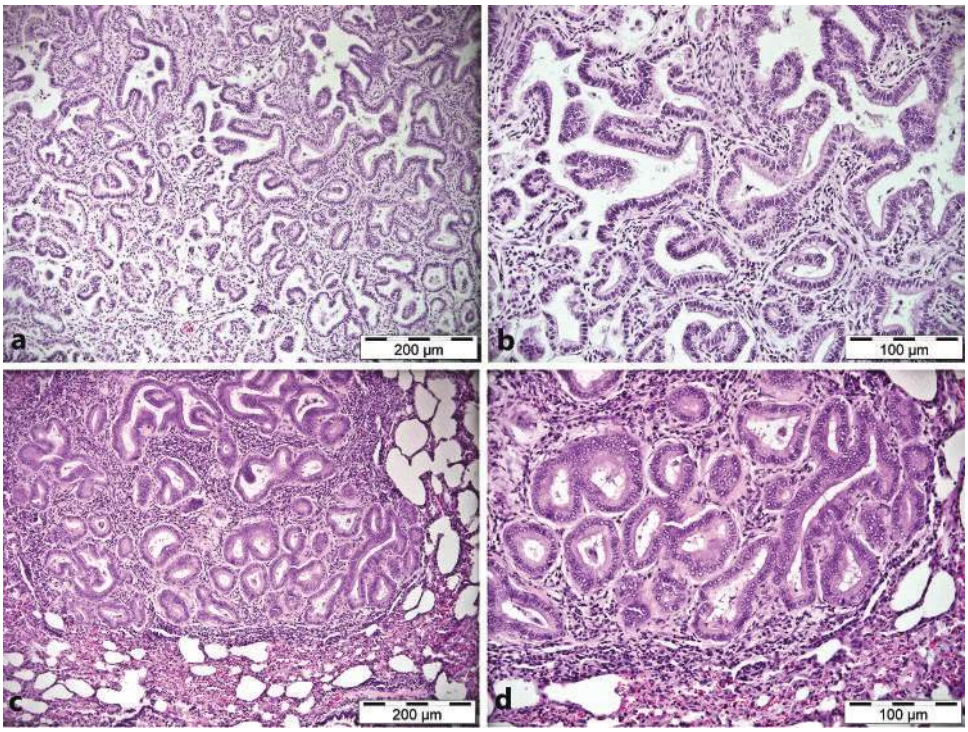

Figure 3. Sheep, OPA, lung tissue, H&E, at different magnifications **a–b:** Classical form, **c–d:** Atypical form

These neoplastic cells formed tumor structures within the alveoli and bronchioles in papillary, acinar, mixed (where papillary and acinar structures occur together) and lepidic patterns. The presence of large and small tumor masses composed of neoplastic cells in both bronchioles and alveoli was noteworthy. While papillary, acinar, or lepidic proliferations were observed singly in some tumor foci, papillary and acinar proliferations were found to occur together in the vast majority of cases (Figures 4 and 5). In addition, the neoplastic cells were well differentiated, with minimal pleomorphism. Mitotic figures were very few.

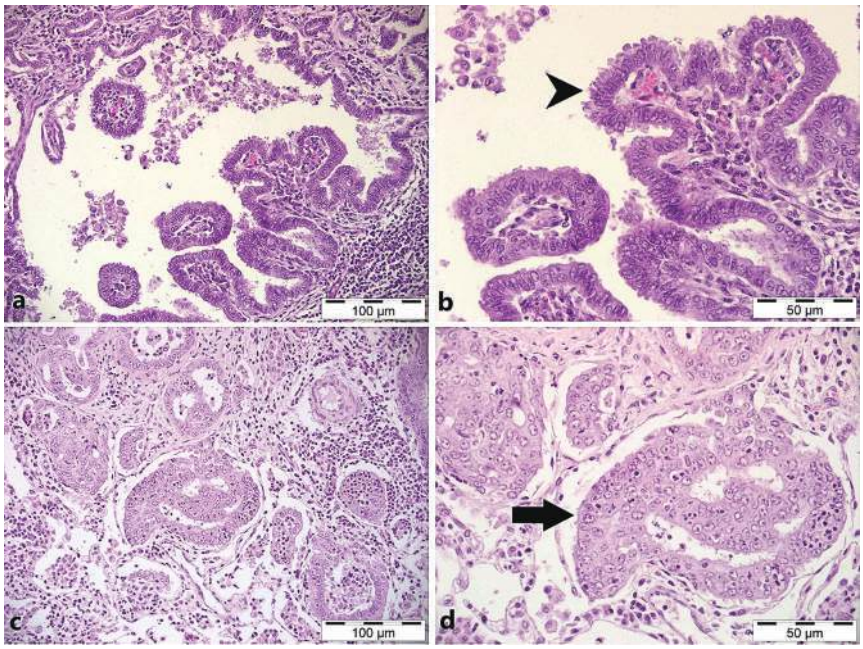

Figure 4. Sheep, OPA, lung tissue, H&E, at different magnifications **a–b:** Papillary proliferations (arrowheads), **c–d:** Acinar tumor structure in the alveolar lumen

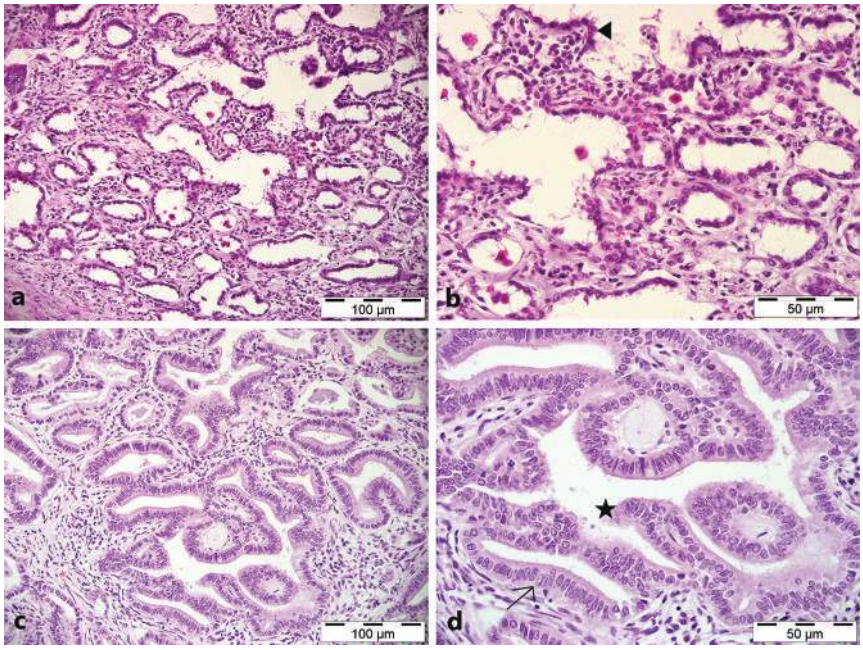

Figure 5. Sheep, OPA, lung tissue, H&E, at different magnifications **a–b:** Lepidic proliferations (arrowheads), **c–d:** Mixed pattern, acinar (thin line) and finger–like projections extending into the lumen (stars)

Inflammatory cell proliferation, predominantly alveolar macrophages, was observed around the cancerous areas. Alveolar macrophages were much more prevalent in the tumor microenvironment in atypical OPA than in the classical form. Interstitial pneumonia, fibrinopurulent bronchopneumonia, and catarrhal purulent bronchopneumonia were also exhibited in association with primary lesions (Figures 6 and 7). In some cases, the inflammatory reaction was so severe that identifying the cancerous areas became particularly challenging.

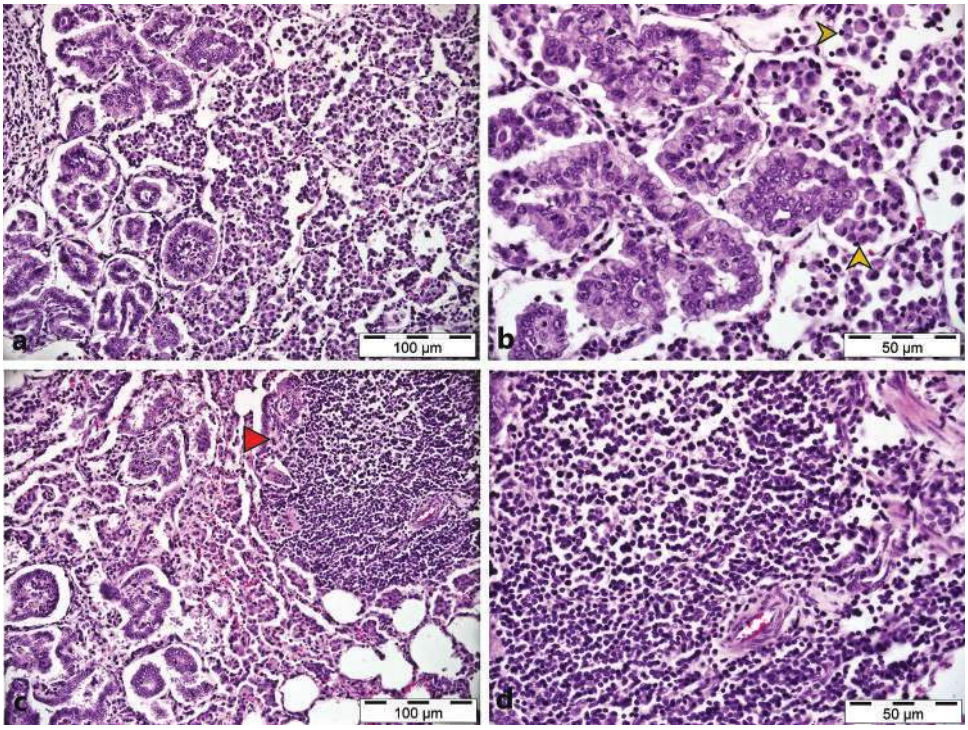

Figure 6. Sheep, OPA, lung tissue, H&E, at different magnifications **a–b:** Very severe alveolar macrophage infiltration in the tumor microenvironment (yellow arrowheads), **c–d:** Lymphoid hyperplasia around the tumoral areas (red arrowhead)

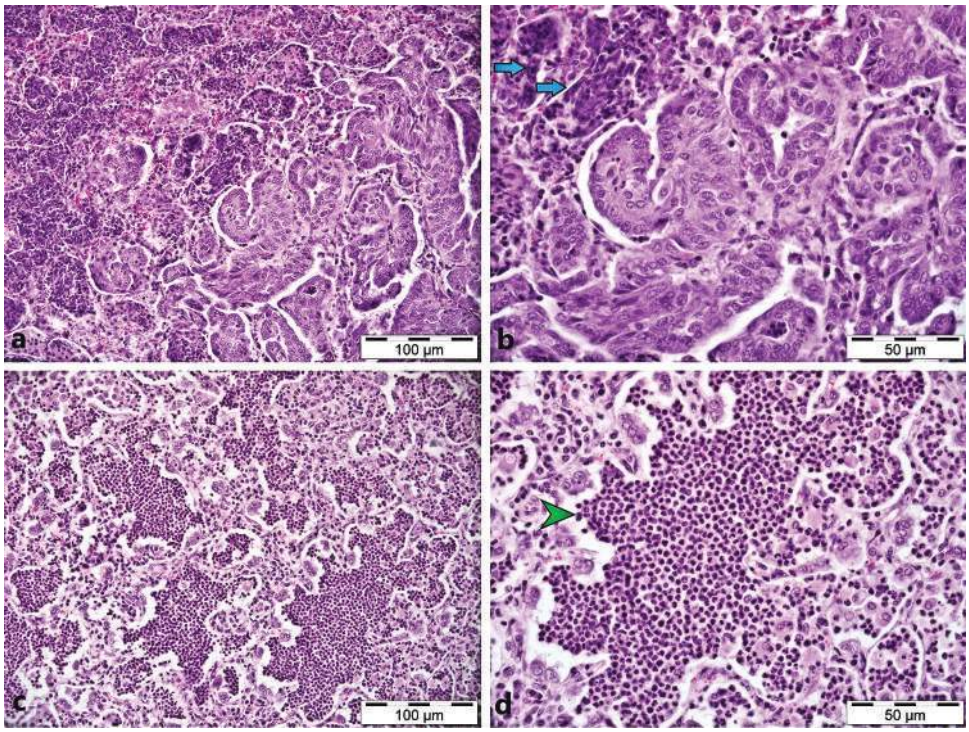

Figure 7. Sheep, OPA, lung tissue, H&E, at different magnifications **a–b:** Fibrinopurulent bronchopneumonia, oat cells (blue arrows) surrounding tumor cells exhibiting papillary growth, **c–d:** Catarrhal purulent bronchopneumonia, very severe neutrophil granulocyte infiltration in the alveolar lumens

As a result of the statistical analysis, the severity of the inflammatory reaction was significantly increased ($P < 0.001$) in JSRV-infected animals compared to the control group (Table 3). However, there was no significant difference between the atypical and classical forms of OPA. The most severe inflammatory findings were predominantly observed in cases of catarrhal and fibrous bronchopneumonia. In addition, a parallel relationship was observed between the presence of alveolar macrophages in the tumor microenvironment, the severity of the inflammatory cell reaction, and tumor aggressiveness. In cases with intense secondary infections, the tumor burden was correspondingly increased, and these cases exhibited a more invasive character, with a tendency to spread throughout the entire lung parenchyma.

Table 3. Inflammation scores of control and infected groups.

	Control (n=10)	Infected (n=10)	P value
Inflammation Scores	0.00 ± 0.00	1.80 ± 1.13	P<0.001

Molecular results

All 10 lung samples tested positive by RT–PCR performed on fresh lung tissues, yielding amplicons of the expected size (398 bp), consistent with the positive control; therefore, the viral etiology of the cases was confirmed (Figure 8).

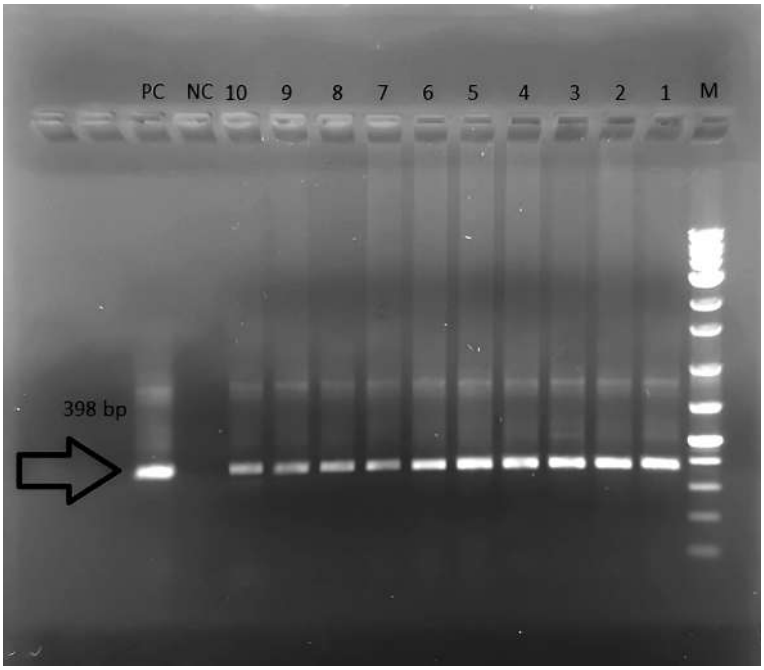

Figure 8. RT–PCR image of the positive samples. **M:** Marker ladder. **PC:** Positive Control, **NC:** Negative Control. The correct product size is 398 base pairs, as shown by the arrow. Wells 1–10 have the corresponding lung tissue samples

Biochemical findings

Homogenate measurements from fresh tissue showed that MDA, NO, ADA, and CP levels were markedly higher ($P < 0.05$) in infected animals compared to the control group, as shown in Table 4. GSH values were statistically significantly lower ($P < 0.001$). No statistical significance was determined for TP and Alb values.

Table 4. Comparison of the concentrations of MDA, NO, GSH, ADA, TP, and ALB in selected sera of OPA–infected sheep and healthy control sheep.

Parameters	Control(n=10)	Infected(n=10)	P value
MDA (μmol/L)	0.42 ± 0.09	1.62 ± 0.24	P<0.001
NO (μmol/L)	0.57 ± 0.14	2.46 ± 0.43	P<0.001
GSH (mg/dL)	4.13 ± 0.53	2.91 ± 0.56	P<0.001
ADA (U/L)	0.82 ± 0.36	1.36 ± 0.34	P<0.05
CP (mg/dL)	0.44 ± 0.13	0.67 ± 0.29	P<0.05
Tp (g/dL)	6.26 ± 0.76	6.37 ± 0.45	NS
Alb (g/dL)	2.51 ± 0.41	2.46 ± 0.38	NS

NS: Not significant

DISCUSSION

Pulmonary adenomatosis infections in sheep resemble human bronchioalveolar adenocarcinoma [7]. No treatment is available for this disease. The disease is usually detected during post–mortem examinations after slaughter. Despite numerous studies on the existence, prevalence, and pathogenesis of this disease, its clinical diagnosis remains incompletely elucidated [24,28]. This study investigated histopathological and biochemical changes in tumor lung tissues.

Previous OPA studies have histopathologically documented the anaplasia and proliferation of alveolar and bronchiolar epithelial cells within pulmonary tissues. Reports also indicate the presence of neutrophil infiltration and alveolar macrophages within the alveolar lumens. Furthermore, mononuclear cell infiltrates have been observed in both interalveolar and peribronchiolar regions [24,28]. In this study, consistent with the extant literature, diffuse tumor foci and an inflammatory response within the lung tissues were detected. In addition, tumor formations were classified into atypical and classical types, similar to the literature [29]. The statistical analyses revealed that the inflammation score was significantly more severe in JSRV-infected animals compared to the control group. In addition, cells within the tumor microenvironment, particularly the presence of alveolar macrophages, were considered to play a substantial role in tumor development. Not only the alveolar macrophages surrounding the tumoral areas, but also the chronicity of the inflammatory reaction and the severity of secondary infections appear to contribute to cancer aggressiveness. In more progressive cases, characterized by diffuse involvement of the entire lung parenchyma rather than isolated tumor foci, the inflammation score was markedly higher, supporting this finding.

Due to its structure, lung tissue is particularly sensitive to ROS [10,24]. In pathological conditions affecting cells or tissues, oxidative stress occurs when the balance favors

ROS over antioxidants. This stress contributes to every stage of carcinogenesis. It has also been recognized as a major factor in the development of lung cancers [30]. Reactive oxygen species (ROS) inflict significant tissue damage [31,32]. Research indicates that ROS are critically involved in the development of tumors [33].

MDA concentrations were observed to be notably elevated in the JSRV-infected group compared to the control group in this study. MDA exhibits high cytotoxicity and the ability to inhibit protective enzymes. Research has shown that oxidative stress and membrane lipid peroxidation play significant roles in the development of various cancers, including lung cancer. Due to this property, MDA is considered a tumor promoter and carcinogenic agent [30,34,35]. It has been reported that free radicals, which play a role in carcinogenesis, may originate from activated phagocytic cells in chronic inflammatory diseases [36]. The present study microscopically demonstrated an increase in phagocytic cells in cancerous areas of the lungs. Furthermore, the identification of catarrhal purulent, fibrinous, and interstitial pneumonia formations in OPA–infected sheep tissues also revealed the presence of inflammation. Inflammation also disrupts the physiological balance in favor of oxidants, creating oxidative stress that can damage cells or tissues [37-40]. In humans, MDA levels have been observed to increase proportionally with advancing cancer stages [31,41]. However, elevated MDA levels have been consistently documented in various lung cancers and across different cancer stages, relative to healthy control groups [33,35,42]. Zalewska–Ziob et al. [10] reported no significant difference in their study. Nevertheless, Karakurt et al.[24] reported an increase in MDA in OPA–infected sheep in their research. In our study, we compared OPA–infected lung tissue with healthy lung parenchyma and found that MDA, an indicator of lipid peroxidation, was increased in JSRV–infected lung tissue. We believe that the increase in MDA is due to the formation of carcinoma tissue and, consequently, to lipid peroxidation associated with inflammation. Additionally, the literature reports increased MDA levels in OPA tissues based on histopathological findings; however, no study was found that evaluated MDA biochemically using tissue homogenates.

Pathogenic agents, chronic irritation, or tissue damage trigger an inflammatory response in cells [43]. Free radicals released by macrophages and leukocytes concentrated at the inflammatory site can damage surrounding tissues and initiate carcinogenesis by altering target molecules and signaling pathways critical for normal physiological homeostasis [44]. NO causes oxidative and nitrosative stress, leading to protein and DNA damage and suppressing the activity of DNA repair enzymes [45]. As a wide variety of reactive oxygen and nitrogen species (ROS/RNS) are produced during chronic inflammation, no clear biomarker has been identified that determines the involvement of a specific reactive species in cancer development [44]. The contribution of chronic inflammation to the development of all types of cancer is widely accepted [43]. High NO levels affect DNA or DNA repair proteins by forming carcinogenic nitrosamines. This effect is genotoxic, leading to increased mutations. Therefore, NO plays a part in the development of cancer cells [46]. NO has also been

found to contribute to lung carcinogenesis in humans [47]. Microscopic examination of OPA cases in this study consistently showed signs of chronic lung inflammation. In this study, NO levels were significantly elevated in the OPA–infected group compared to the healthy control group. The presence of high levels of inflammatory mediators in infected lungs may promote the release of free radicals; similarly, elevated NO levels may create a microenvironment that supports tumor formation or heterogeneity, potentially leading to metastasis, which may explain its increased levels in OPA–infected sheep.

Ceruloplasmin is an inflammatory reactive protein. It enables the clearance of oxygen radicals. In addition, CP has been linked to the development and progression of tumors. Increased concentrations of CP have been documented in pulmonary carcinomas and other cancer types [14]. Additionally, CP levels increase in conditions such as iron deficiency, pregnancy, and inflammatory states [48,49]. While CP is mainly synthesized by the liver, lung adenocarcinoma cells have demonstrated the capacity for its heterotopic production and secretion [14,50]. No studies on CP in animals with OPA were found in the literature search. However, its elevation in human lung adenocarcinomas has been reported in previous studies [14,51]. In our study, we believe that the significantly higher CP levels in OPA–affected sheep lung tissues compared to the control group are due to CP production by lung adenocarcinoma cells. Furthermore, the increase in CP may be caused by OPA triggering oxidative stress in lung tissue.

Reactive oxygen species and imbalances in the antioxidant barrier regulate the tumor formation process. GSH has an important role in protecting cells from oxidative damage by clearing ROS. An imbalance between ROS and antioxidants in cells leads to pathological conditions. Increased ROS and insufficient antioxidant defense against oxidative products have been reported to promote the initiation of lung adenocarcinoma and various other tumors [52-54]. Despite antioxidant–mediated ROS balance, excessive ROS accumulation can cause cell cycle arrest, accelerate cell ageing, and promote apoptosis [55]. Oxidative damage is prevented by GSH redox homeostasis. It is known that a decrease in GSH levels or prolonged low levels renders mammalian cells susceptible to DNA damage [57]. Hydroperoxides, through DNA damage, are likely to act antagonistically against GSH in the early stages of carcinogenesis [11,57,58]. The fact that Humann–Ziehank et al. [59] found significant decreases in glutathione peroxidases in animals with OPA aligns with the findings of our study. In our study, we attributed the significant reduction in GSH levels in tissues compared to healthy tissues to the consumption of GSH required to neutralize the excessive ROS produced by tumor cells.

ADA, a pivotal enzyme involved in purine metabolism, catalyzes the hydrolytic deamination of adenosine into inosine and deoxyadenosine into deoxyinosine, thereby reducing adenosine, which is an important immunosuppressive signal [60]. Adenosine confers tumor resistance to the immune system. It accumulates in tumors, promoting tumor growth and angiogenesis [61]. ADA also plays an essential role in many

physiological reactions, such as neurological, immunological, and vascular processes. ADA plays an important role in the proliferation and differentiation of lymphocytes, as well as in the maturation of the immunological system [62,63]. The maintenance of T-lymphocyte function is dependent on ADA activity. Therefore, the ADA enzyme is essential for the immune response. ADA, which at normal levels has anti-inflammatory effects, is considered a marker of T–cell activation and inflammation. Its high activity has been reported to cause tissue and organ damage [64-66]. ADA is an enzyme secreted by macrophages and activated T-lymphocytes [60]. In OPA cases, marked macrophage and lymphocyte infiltration observed in histopathological examinations of tumor regions may suggest increased ADA production to break down adenosine. Previous studies in human and veterinary medicine have shown that ADA activity changes in both neoplastic and non-neoplastic pathological conditions [63,67-69]. The marked increase in ADA activity detected in our study is considered to be associated with the increased demand for purine nucleotides by rapidly proliferating neoplastic tissue, together with severe hypoxia and necrosis developing during OPA pathogenesis, or with chronic inflammation and intense T-cell proliferation in the alveolar spaces. Although these findings indicate that ADA may play a critical role in the immunopathogenesis of the disease, the fact that the sample group was not compared with different lung pathologies limits the direct identification of this enzyme as a specific diagnostic biomarker. Therefore, ADA should currently be regarded as a potential candidate marker for understanding the mechanisms underlying OPA development, and its diagnostic sensitivity and specificity should be supported by future large-scale clinical studies and advanced statistical analyses.

Consequently, OPA cannot be diagnosed in the early stages of the disease because it does not present clinical symptoms. This study found, by both biochemical and histopathological examinations, that oxidative stress occurs in the lung tissues of OPA–infected sheep and that ADA activity increases. Furthermore, this study is the first to determine MDA levels in lung tissue supernatants from cases diagnosed with OPA using biochemical methods. Moreover, no study examining ADA activity in OPA was identified in the literature review; therefore, this study provides a novel contribution to the literature. As there are no treatment or prevention procedures for OPA and infected animals must be rapidly removed from herds, further studies are needed to support clinical findings and to determine the pathophysiology in detail. In addition, alveolar macrophage–mediated inflammatory reactions and the presence of secondary infections within the tumor microenvironment appear to increase the tumor burden and contribute significantly to the progression of OPA.

Authors' contributions

ŞK contributed to conceptualization, methodology, sample collection, coordination, and drafting and writing of the manuscript. EK performed histopathological analyses and contributed to the writing of the manuscript. GE contributed to the study design and sample collection. NC carried out the execution of molecular analyses. SG

performed histopathological stainings. ÇO performed biochemical tests and statistical analysis. OM performed biochemical tests. All authors have read and approved the final version of the manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ORCID iDs

Şemistan Kızıltepe https://orcid.org/0000-0003-3727-8893
Emin Karakurt https://orcid.org/0000-0003-2019-3690
Gizem Eser https://orcid.org/0000-0002-8330-3095
Nüvit Coşkun https://orcid.org/0000-0001-7642-6460
Selda Güneş https://orcid.org/0009-0008-8569-2064
Çağlar Okulmuş https://orcid.org/0000-0003-3563-3716
Oğuz Merhan https://orcid.org/0000-0002-3399-0667

REFERENCES

1. De las Heras M, Ortin A, Cousens C, Minguijon E, Sharp JM: Enzootic nasal adenocarcinoma of sheep and goats. In: Jaagsiekte sheep retrovirus and lung cancer. 2003. p. 201-223.
2. Coşkun N, Yilmaz V, Karakurt E, Beytut E, Nuhoglu H, Timurkan MÖ: Molecular and pathological detection of Jaagsiekte sheep Retrovirus in lung tissues of sheep. Kafkas Üniversitesi Veteriner Fakültesi Dergisi 2024, 30(6):809-814.
3. Johnson C, Fan H: Jaagsiekte Sheep Retrovirus and Lung Cancer. In: Robertson E, editor. Cancer Associated Viruses. Boston (MA): Springer; 2012.
4. Ortin A, De las Heras M, Borobia M, Ramo MA, Ortega M, de Arcaute MR: Ovine pulmonary adenocarcinoma: A transmissible lung cancer of sheep, difficult to control. Small Rumin Res 2019, 176:37-41.
5. De las Heras M, Borobia M, Ortín A: Neoplasia-associated wasting diseases with economic relevance in the sheep industry. Animals 2021, 11(2):381.
6. Larruskain A, Esparza-Baquer A, Minguijón E, Juste RA, Jugo BM: SNPs in candidate genes MX dynamin-like GTPase and chemokine (C-C motif) receptor-5 are associated with ovine pulmonary adenocarcinoma progression in Latxa sheep. Animal Genetics 2015, 46(6):666-675.
7. Larruskain A, Minguijón E, Arostegui I, Moreno B, Juste RA, Jugo BM: Microsatellites in immune-relevant regions and their associations with Maedi-Visna and ovine pulmonary adenocarcinoma viral diseases. Vet Immunol Immunopathol 2012, 145(1-2):438-446.
8. Gray ME, Meehan J, Sullivan P, Marland JR, Greenhalgh SN, Gregson R, Clutton RE, Ward C, Cousens C, Griffiths DJ, Murray A, Argyle D: Ovine pulmonary adenocarcinoma: a unique model to improve lung cancer research. Front Oncol 2019, 9:335.
9. Gào X, Holleczek B, Cuk K, Zhang Y, Anusruti A, Xuan Y, Xu Y, Brenner H, Schöttker B: Investigation on potential associations of oxidatively generated DNA/RNA damage with lung, colorectal, breast, prostate and total cancer incidence. Sci Rep 2019, 9(1):7109.

10. Zalewska-Ziob M, Adamek B, Kasperczyk J, Romuk E, Hudziec E, Chwalińska E, Dobija-Kubica K, Rogoziński P, Bruliński K: Activity of antioxidant enzymes in the tumor and adjacent noncancerous tissues of nonsmall-cell lung cancer. Oxid Med Cell Longev 2019, 2019:2901840.
11. Hayes JD, Dinkova-Kostova AT, Tew KD: Oxidative stress in cancer. Cancer Cell 2020, 38(2):167–197.
12. Yarım GF, Nisbet C, Çenesiz S, Coşkuner A: Şap hastalıklı koyunlarda serum nitrik oksit düzeyi ve adenozin deaminaz aktivitesinin araştırılması. Ankara Üniversitesi Veteriner Fakültesi Dergisi 2006, 53(3):161-164.
13. Choudhari SK, Chaudhary M, Bagde S, Gadbail AR, Joshi V: Nitric oxide and cancer: a review. World J Surg Onc 2013, 11:118.
14. Matsuoka R, Shiba-Ishii A, Nakano N, Togayachi A, Sakashita S, Sato Y, Minami Y, Noguchi M: Heterotopic production of ceruloplasmin by lung adenocarcinoma is significantly correlated with prognosis. Lung Cancer 2018, 118:97–104.
15. Bozukluhan K, Merhan O, Kızıltepe Ş, Harmankaya A, Gökçe G: Determination of oxidative stress and ceruloplasmin levels in sheep with toxoplasmosis. Van Veterinary Journal 2020, 31(2).
16. Chen F, Han B, Meng Y, Han Y, Liu B, Zhang B, Chang Y, Cao P, Fan Y, Tan K: Ceruloplasmin correlates with immune infiltration and serves as a prognostic biomarker in breast cancer. Aging (Albany NY) 2021, 13(16):20438.
17. Patil MB, Lavanya T, Kumari CM, Shetty SR, Gufran K, Viswanath V, Swarnalatha C, Babu JS, Nayyar AS: Serum ceruloplasmin as cancer marker in oral pre-cancers and cancers. J Carcinog 2021, 20:15.
18. Bagheri S, Saboury AA, Haertlé T: Adenosine deaminase inhibition. Int J Biologic Macromolecul 2019, 141:1246-1257.
19. Sonawane GG, Tripathi BN, Kumar R, Kumar J: Diagnosis and prevalence of ovine pulmonary adenocarcinoma in lung tissues of naturally infected farm sheep. Vet World 2016, 9(4):365.
20. Yoshioka T, Kawada K, Shimada T, Mori M: Lipid peroxidation in maternal and cord blood and protective mechanism aganist activated-oxygen toxicity in the blood. Am J Obstet Gynecol 1979, 135(3):372-376.
21. Miranda KM, Espey MG, Wink DA: A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide: Biol Chem 2001, 5(1):62-71.
22. Beutler E, Duron O, Kelly BM: Improved method for determination of blood glutathione. J Lab Clin Med 1963, 61:882-888.
23. Colombo JP, Richterich R: Zur bestimmung des caeruloplasmin im plasma. Schweiz Med Wochenschr 1964, 23:715-720.
24. Karakurt E, Beytut E, Dağ S, Nuhoğlu H, Yıldız A, Kurtbaş E: Assessment of MDA and 8-OHdG expressions in ovine pulmonary adenocarcinomas by immunohistochemical and immunofluorescence methods. Acta Vet Brno 2022, 91(3): 235-241.
25. Karakurt E, Coşkun N, Beytut E, Keleş ÖF, Dağ S, Yılmaz V, Nuhoğlu H, Yıldız A, Kurtbaş E: Evaluation of the relationship between inflammatory reaction and interleukins in ovine pulmonary adenocarcinomas. Vet Res Forum 2023, 14(1):1-6.
26. Chomczynski P, Sacchi N: The single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction: twenty-something years on. Nature protocols 2006, 1(2):581-585.

27. Mansour KA, Al-Husseiny SH, Kshash QH, Jassim A: Clinical-histopathological and molecular study of ovine pulmonary adenocarcinoma in Awassi sheep in Al-Qadisiyah Province, Iraq. Vet World 2019: 12(3):454.
28. İlhan F, Vural SA, Yıldırım S, Sözdutmaz İ, Alcigir ME: Expression of p53 protein, Jaagsiekte sheep retrovirus matrix protein, and surfactant protein in the lungs of sheep with pulmonary adenomatosis. J Vet Diagnost Invest 2016, 28(3):249-256.
29. Karakurt E, Coşkun N, Keleş ÖF, Beytut E, Yaman T, Uysal AY, Güneş S, Çiçek H A: Comparison of classical and atypical forms of ovine pulmonary adenocarcinomas using ımmunohistochemical and molecular techniques. Pakistan Vet J 2025, 45(4):1744-1754.
30. Kaynar H, Meral M, Turhan H, Keles M, Celik G, Akcay F: Glutathione peroxidase, glutathione-S-transferase, catalase, xanthine oxidase, Cu-Zn superoxide dismutase activities, total glutathione, nitric oxide, and malondialdehyde levels in erythrocytes of patients with small cell and non-small cell lung cancer. Cancer Lett 2005, 227:133-139.
31. Srivastava AN, Gupta A, Srivastava S, Natu SM, Mittal B, Negi MP, Prasad R: Cisplatin combination chemotherapy induces oxidative stress in advance non small cell lung cancer patients. Asian Pac J Cancer Prev 2010, 11:465-471.
32. Kızıltepe S, Okulmuş Ç, Merhan O, Mor N, Uzlu E: Determination of malondialdehyde, nitric oxide, reduced glutathione, sialic acid and ceruloplasmin levels in sheep liver tissue with hydatic cyst. Kocatepe Vet J 2024, 17(1):55-61.
33. Sunnetcioglu A, Alp HH, Sertogullarından B, Balaharoglu R, Gunbatar H: Evaluation of oxidative damage and antioxidant mechanisms in copd, lung cancer, and obstructive sleep apnea syndrome. Respir Care 2016, 61:205-211.
34. Esme H, Cemek M, Sezer M, Saglam H, Demir A, Melek H, Unlu M: High levels of oxidative stress in patients with advanced lung cancer. Respirology 2008, 13:112-116.
35. Otsmane A, Kacimi G, Adane S, Cherbal F, Aouichat Bouguerra S: Clinico-epidemiological profile and redox imbalance of lung cancer patients in Algeria. J Med Life 2018, 11:210-217.
36. Zieba M, Suwalski M, Kwiatkowska S, Piasecka G, Grzelewska–Rzymowska I, Stolarek R, Nowak D: Comparison of hydrogen peroxide generation and the content of lipid peroxidation products in lung cancer tissue and pulmonary parenchyma. Resp Med 2000, 94(8):800-805.
37. Ergönül S: Anaplasmosisli sığırlarda ısı şok protein (HSP), malondialdehit (MDA), nitrik oksit (NO) ve interlökin (IL-6, IL-10) düzeylerinin araştırılması. Kafkas Üniversitesi Veteriner Fakültesi Dergisi 2009;15(4).
38. Bozukluhan K, Merhan O, Öğün M, Cihan M, Gökçe G: Omfalitisli buzağılarda bazı oksidatif stres parametre düzeylerinin belirlenmesi. F Ü Sağ Bil Vet Derg 2016, 30(2):79-81.
39. Xu W, Zhao T, Xiao H: The implication of oxidative stress and AMPK-Nrf2 antioxidative signaling in pneumonia pathogenesis. Front Endocrinol 2020, 11:400.
40. Shabestari AA, Imanparast F, Mohaghegh P, Kiyanrad H: The effects of asthma on the oxidative stress, inflammation, and endothelial dysfunction in children with pneumonia. BMC Pediatrics 2022, 22(1):534.
41. Peddireddy V, Siva Prasad B, Gundimeda SD, Penagaluru PR, Mundluru HP: Assessment of 8-oxo-7, 8-dihydro-2'-deoxyguanosine and malondialdehyde levels as oxidative stress markers and antioxidant status in non-small cell lung cancer. Biomarkers 2012, 17:261-268.
42. Gęgotek A, Nikliński J, Žarković N, Žarković K, Waeg G, Łuczaj W, Charkiewicz R, Skrzydlewska E: Lipid mediators involved in the oxidative stress and antioxidant defence of human lung cancer cells. Redox Biol 2016, 9:210-219.

43. Coussens L, Werb Z: Inflammation and cancer. Nature 2002, 420:860–867.
44. Muntané J, De la Mata M: Nitric oxide and cancer. World J Hepatol 2010, 2(9):337.
45. Hofseth LJ, Hussain SP, Wogan GN, Harris CC: Nitric oxide in cancer and chemoprevention. Free Radic Biol Med 2003, 34:955–968.
46. Kaplan İ, Aydın Y, Bilen Y, Genç F, Keleş MS, Eroğlu A: The evaluation of plasma arginine, arginase, and nitric oxide levels in patients with esophageal cancer. Turkish J Med Sci 2012, 42(3):403-409.
47. Masri FA, Comhair SA, Koeck T, Xu W, Janocha A, Ghosh S, Dweik RA, Golish J, Kinter M, Stuehr DJ, Erzurum SC, Aulak KS: Abnormalities in nitric oxide and its derivatives in lung cancer. Am J Respir Crit Care Med 2005, 172(5):597-605.
48. Pejovic M, Djordjevic V, Ignjatovic I, Stamenic T, Stefanovic V: Serum levels of some acute phase proteins in kidney and urinary tract urothelial cancers. Int Urol Nephrol 1997, 29:427-432.
49. Varela AS, Saez J-BL, Senra DQ: Serum ceruloplasmin as a diagnostic marker of cancer. Cancer Letters 1997, 121(2):139-145.
50. Wang KK, Liu N, Radulovich N, Wigle DA, Johnston MR, Shepherd FA, Minden MD, Tsao MS: Novel candidate tumor marker genes for lung adenocarcinoma. Oncogene 2002, 21(49):7598–7604.
51. Chang WM, Li LJ, Chiu IA, Lai TC, Chang YC, Tsai HF, Yang CJ, Huang MS, Su CY, Lai TL, Jan YH, Hsiao M: The aberrant cancer metabolic gene carbohydrate sulfotransferase 11 promotes non-small cell lung cancer cell metastasis via dysregulation of ceruloplasmin and intracellular iron balance. Transl Oncology 2022, 25:101508.
52. Neumann CA, Krause DS, Carman CV, Das S, Dubey DP, Abraham JL, Bronson RT, Fujiwara Y, Orkin SH, Van Etten RA: Essential role for the peroxiredoxin Prdx1 in erythrocyte antioxidant defence and tumor suppression. Nature 2003, 424(6948):561–565.
53. Gill JG, Piskounova E, Morrison SJ: Cancer, Oxidative Stress, and Metastasis. Cold Spring Harb Symp Quant Biol 2016, 81:163–175.
54. Canli Ö, Nicolas AM, Gupta J, Finkelmeier F, Goncharova O, Pesic M, Neumann T, Horst D, Löwer M, Sahin U, Greten FR: Myeloid cell-derived reactive oxygen species ınduce epithelial mutagenesis. Cancer Cell 2017, 32(6):869–883.e5.
55. Liou GY, Storz P: Reactive oxygen species in cancer. Free Radic Res 2010, 44(5):479–496.
56. Baliga MS, Diwadkar-Navsariwala V, Koh T, Fayad R, Fantuzzi G, Diamond AM: Selenoprotein deficiency enhances radiation-induced micronuclei formation. Mol Nutr Food Res 2008, 52(11):1300-1304.
57. Shi ZZ, Osei-Frimpong J, Kala G, Kala SV, Barrios RJ, Habib GM, Lukin DJ, Danney CM, Matzuk MM, Lieberman MW: Glutathione synthesis is essential for mouse development but not for cell growth in culture. Proc Natl Acad Sci USA 2000, 97(10):5101–5106.
58. Galadari S, Rahman A, Pallichankandy S, Thayyullathil F: Reactive oxygen species and cancer paradox: To promote or to suppress? Free Radic Biol Med 2017, 104:144–164.
59. Humann-Ziehank E, Renko K, Bruegmann ML, Devi VR, Hewicker-Trautwein M, Andreae A, Ganter M: Long-term study of ovine pulmonary adenocarcinogenesis in sheep with marginal vs. sufficient nutritional selenium supply: results from computed tomography, pathology, immunohistochemistry, JSRV-PCR and lung biochemistry. J Trace Elem Med Biol 2013, 27(4):391-399.

60. Hocanlı İ, Uzer F, Çil B, Kırhan İ, Günak F: Diagnostic value of adenosine deaminase in bronchoalveolar lavage fluid for patients with lung cancer. Int J Clin Pract 2021, 75(12):e14918.
61. Linden J: Adenosine metabolism and cancer. Focus on "Adenosine downregulates DPPIV on HT-29 colon cancer cells by stimulating protein tyrosine phosphatases and reducing ERK1/2 activity via a novel pathway". Am J Physiol Cell Physiol 2006, 291(3):C405-C406.
62. Flinn AM, Gennery AR: Adenosine deaminase deficiency: a review. Orphanet J Rare Dis 2018, 13(1):65.
63. Kahraman MA, Gökçe Hİ: Investigations of Adenosine Deaminase and C-reactive Protein in Cats with Feline Infectious Peritonitis. Mehmet Akif Ersoy University Journal Of Health Sciences Institute 2020, 8(3):98-107.
64. Baba K, Hoosen AA, Langeland N, Dyrhol-Riise AM: Adenosine deaminase activity is a sensitive marker for the diagnosis of tuberculous pleuritis in patients with very low CD4 counts. PLoS One 2008, 3(7):e2788.
65. Brigida I, Sauer AV, Ferrua F, Giannelli S, Scaramuzza S, Pistoia V, Castiello MC, Barendregt BH, Cicalese MP, Casiraghi M, Brombin C, Puck J, Müller K, Notarangelo LD, Montin D, van Montfrans JM, Roncarolo MG, Traggiai E, van Dongen JJ, van der Burg M, Aiuti A: B-cell development and functions and therapeutic options in adenosine deaminase–deficient patients. J Allergy Clin Immunol 2014, 133(3):799-806.
66. Niraula A, Thapa S, Kunwar S, Lamsal M, Baral N, Maskey R: Adenosine deaminase activity in type 2 diabetes mellitus: does it have any role? BMC Endocr Disord 2018, 18:58.
67. Salmanzadeh S, Tavakkol H, Bavieh K, Alavi SM: Diagnostic value of serum adenosine deaminase (ADA) level for pulmonary tuberculosis. J Jundishapur Microbiol 2015, 8(3): e21760.
68. Sarıkaya S, Gökce Hİ: Investigations of adenosine deaminase 1, paraoxonase 1, C-reactive protein and serum amyloid A levels in cats infected with calicivirus. MeditVetJ 2021, 6(3):133-138.
69. Kızıltepe S, Okulmuş Ç, Akpınar R, Merhan O, Bozukluhan K, Coşkun N: Investigation of Adenosine deamidase (ADA) activity and some biochemical values in lambs with infectious ecthyma symptoms. Arq Bras Med Vet Zootec 2025, 77(04):e13399.

OKSIDATIVNI STRES I AKTIVNOST ADENOZIN DEAMINAZE KOD OVACA SA ADENOKARCINOMOM PLUĆA

Şemistan KIZILTEPE, Emin KARAKURT, Gizem ESER,
Nüvit COŞKUN, Selda GÜNEŞ, Çağlar OKULMUŞ, Oğuz MERHAN

Pulmonarni adenokarcinom ovaca (OPA) je infektivna i neoplastična bolest etiološki povezana sa retrovirusom ovaca Jaagsiekte (JSRV), koju karakterišu tumorske lezije pluća. Zbog ekonomskih gubitaka koje izaziva, OPA je od velikog značaja za zdravlje stada. U ovoj studiji, markeri oksidativnog stresa i aktivnost adenozin deaminaze (ADA) kvantifikovani su u plućnom tkivu ovaca, kako zdravih tako i onih prirodno obolelih od OPA. U poređenju sa zdravim ovcama, koncentracije/aktivnosti malon-

dialdehida (MDA), azot-oksida (NO), ceruloplazmina (CP) i ADA bile su značajno povećane ($P<0,05$ i $P<0,001$) u svežem plućnom tkivu ovaca zaraženih JSRV-om, dok su nivoi redukovanog glutationa (GSH) bili značajno niži ($P<0,05$). Možemo da zaključimo da kod ovaca zaraženih JSRV-om otkriveni su izražen oksidativni stres i povećana aktivnost ADA enzima. Ovi nalazi ukazuju na to da bi ADA aktivnost mogla poslužiti kao biomarker za dijagnozu bolesti.