

## Determination of Telomerase Enzyme Level in Enzootic Intranasal Tumors of Goats

Şima ŞAHİNDURAN<sup>1,a</sup> Özlem ÖZMEN<sup>2,b</sup> Reyda KIYICI<sup>1,c</sup>

<sup>1</sup> Mehmet Akif Ersoy Üniversitesi, Veteriner Fakültesi, İç Hastalıkları Anabilim Dalı, TR-15030 Burdur - TÜRKİYE

<sup>2</sup> Mehmet Akif Ersoy Üniversitesi, Veteriner Fakültesi, Patoloji Anabilim Dalı, TR-15030 Burdur - TÜRKİYE

<sup>a</sup> ORCID: 0000-0002-7718-2020; <sup>b</sup> ORCID: 0000-0002-1835-1082; <sup>c</sup> ORCID: 0000-0002-0667-5477

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### Abstract

Enzootic intranasal adenocarcinoma (ENA) of goats are contagious tumoral disease that characterized occurrence of tumoral masses in nasal cavity of the animals. The etiological agent of the disease is retrovirus and the disease cause high economical loses in goat industry. Retroviruses, respectively inducing lung or nasal adenocarcinomas. The true economic impact of ENA is not known because affected animals are given insights from the herd before actual diagnosis, and suspected disease incidence is rarely reported. Telomerase regulates the proliferative capacity of cells and this enzyme has critical role in tumor progression. The aim of this study is to evaluate telomerase activity in serum and tumoral tissue in goat with ENA. For this aim 11 goat's serum were analyses for telomerase activity. Tumoral tissue sections of totally 14 death goats were examined by immunohistochemically for telomerase expression. In addition, 10 normal goat's serum and nasal tissues that belong slaughtered healthy animals used as control. This study showed that increased serum and tissue telomerase activity in goats with ENA comparing to controls. Also our results showed that telomerase activity becomes a useful prediction marker for tumor development in infected but clinically healthy goats in infected flocks.

**Keywords:** Enzootic intranasal tumor, ELISA, Immunohistochemistry, Telomerase

## Keçilerin Enzootik Intranazal Tümörlerinde Telomeraz Enzim Düzeyinin Belirlenmesi

### Öz

Keçilerin enzootik intranazal adenokarsinomu (ENA), hayvanların burun boşluğunda tümöral kitlelerin şekillenmesi ile karakterize bulaşıcı bir hastalıktır. Hastalığın etkeni retrovirüs olup, keçi endüstrisinde ekonomik kayıplara neden olmaktadır. Retroviruslar sırasıyla akciğer veya nazal adenokarsinomlara yol açar. Hastalıktaki ekonomik kayıp tam olarak bilinmemektedir. Çoğu zaman hasta hayvanlar teşhisten önce sürüden çıkarıldıkları için hastalığın gerçek insidansı ile ilgili bildirimler nadirdir. Telomeraz, hücrelerin proliferatif kapasitesini düzenler ve bu enzim tümörün yayılmasında kritik bir role sahiptir. Bu çalışmanın amacı ENA'lı keçilerin kan serumu ve tümöral dokularında telomeraz aktivitesini değerlendirmektir. Bu amaçla, 11 ENA'lı keçi serumunda telomeraz aktivitesi için analizler yapıldı. Ayrıca toplam 14 ölü keçinin tümöral doku kesitleri, telomeraz ekspresyonu için immunohistokimyasal olarak incelendi. Kontrol olarak, kesimi yapılan 10 sağlıklı keçinin serumu ve burun dokusu kullanıldı. Bu çalışmada ENA'lı keçilerde serum ve tümör dokularında telomeraz aktivitesinin kontrol grubundaki keçilere kıyasla arttığı tespit edildi. Enfekte olmuş fakat klinik olarak sağlıklı görünen keçilerde telomeraz aktivitesinin tümör gelişimi için yararlı bir belirteç olabileceği görüldü.

**Anhtar sözcükler:** Enzootik intranazal tümör, ELISA, İmmunohistokimya, Telomeraz

## INTRODUCTION

Telomerase activity is an important determinant of telomere length in mammalian cells, in which lack of telomerase activity could exacerbate cell senescence,

especially in highly proliferative tissues<sup>[1,2]</sup>. Telomerase activity has been shown to be specifically expressed in immortal cells, cancer and germ cells where it compensates for telomere shortening during DNA replication and thus stabilizes telomere length<sup>[3]</sup>.



### İletişim (Correspondence)



+90 248 2132202



sahinduran@mehmetakif.edu.tr

Telomere shortening during the cell division disturbed tumor progression. Because of the continuous cell growth in malign tumors telomerase activity correlated by tumor behavior [4]. Telomerase is a cellular reverse transcriptase and adds new DNA onto the telomeres that are located at the ends of chromosomes [4-6]. Expression of telomerase is associated with the stage of differentiation and inhibition or absence of telomerase may result in cell crisis in cancer cells and cause tumor regression [7]. In addition, telomerase may express in permanently renewing epithelia such as gastrointestinal tract [8]. Shortening of telomeres may contribute to the control of the proliferative capacity in normal cells, and telomerase may be essential for unlimited cell proliferation [9]. An ideal cancer treatment would specifically target cancerous cells and have little or no effect on normal cells. Because of the compelling correlation that most normal cells not expressed telomerase and activity is detected in almost all cancer types, it may be a universal target for cancer therapeutics. Telomerase-based therapies should possess greater specificity, lower toxicity, and reduced side effects compared to conventional chemotherapeutic approaches [10,11].

Many viruses have been shown to be able to increase telomerase activity, for example the LMP1 gene of Epstein Barr virus (EBV) [12], Kaposi's sarcoma herpesvirus [13], Marek's disease virus [14], herpes simplex virus type-1 (HSV-1) [15] and Bovine Herpesvirus type1 -all up-regulate telomerase activity. On the other hands some viruses such as human immunodeficiency virus [16], the LMP2A gene of Epstein Barr virus [17] and Hepatitis B virus [18] down-regulate telomerase activity.

Sheep and goats are widely infected by the non-oncogenic Small Ruminant LentiViruses, related to Human Immunodeficiency Virus-1 and responsible for slowly evolving inflammatory and/or degenerative diseases, and by oncogenic retroviruses, and Enzootic Nasal Tumour Virus (ENTV), respectively inducing lung or nasal adenocarcinomas [19,20]. Enzootic nasal adenocarcinoma (ENA) is an economically important contagious tumor of the nasal mucosa in sheep and goats [21]. The true economic impact of ENA is not known because affected animals are given insights from the herd before actual diagnosis, and suspected disease incidence is rarely reported [22]. Although the exact prevalence of ENA is unknown [23] and often is not diagnosed.

The agent of ENA is a simple retrovirus and it induces unilateral or bilateral neoplastic growth in the mucosal nasal glands of the ethmoidal area [21]. No metastasis has been reported in ENA cases, but disruption of the nasal septum structure as well as erosion of the cribiform plate has been reported [23,24].

Clinical signs of ENA include seromucosal nasal discharge leading to a 'washed nose' appearance, accompanied by snoring, coughing, wheezing and dyspnea. The duration

of disease, from the appearance of clinical signs to the time of death, varies from 3 weeks to 1 year or more.

Based on these findings, we investigated the relationship between telomerase value in serum and immuno expression in tissue in enzootic nasal adenocarcinoma in goats.

## MATERIAL and METHODS

This study was carried out on a total of eleven (5 males and 6 females) alive and 3 died (all of female) goats (*Capra hircus*) from a herd numbering 225 goats with a 10-year history of respiratory nasal problems and death with respiratory distress. The goats kept in a small shelter together and they ages were different between 2 to 5 years old.

The farm was visited and blood samples from 11 clinically ill animals (infected group=11) collected for evaluate the serum levels of telomerase. Clinically, signs of dyspnea and mucous nasal discharge were observed in many of the animals. Mild to severe emaciation was noted in all of animals. According to the history that taken from owner, more than 120 goats died from same illness with dyspnea and in some cases nasal bone perforation in recent years. Blood samples were collected in tubes without anticoagulant and were centrifuged at 3000 rpm, at 4°C for 10 min. Furthermore, ten healthy goats (control group=10) were used from another healthy flock. Serum samples were carefully harvested and stored at -20°C until used. These sera were then used to establish the values of telomerase levels using an ELISA commercial kits [Goat Telomerase (TE) ELISA KIT].

The 11 alive goats with clinical symptoms (blood samples collected from) were euthanatized, in addition 3 death goats were underwent to necropsy. At the gross examination of nasal cavity, unilateral intranasal tumors were found in all 14 goats. During necropsy, tissue samples were taken from tumoral masses for histopathological examinations. Samples were fixed in 10% neutral formalin. Using standard methods, tissues were stained with Hematoxylin-Eosin (HE), and examined microscopically.

Selected tumor sections were stained immunohistochemically in order to demonstrate telomerase activity in tumor tissue [Anti-Telomerase reverse transcriptase antibody -C-terminal ab183105, (1/100 dilution)] using a routine streptavidin biotin peroxidase technique. For immunohistochemical examination sections were routinely processed according the manufacturer's instructions.

Student t test were used for to evaluate the differences of serum telomerase activity between the goats with and without ENA Variables were presented as, "mean±standard deviation" deviations. Calculations were made using the SPSS 15.0 program pack (SPSS Inc., Chicago, IL, USA). P<0.05 was set as the value for significance.

## RESULTS

### Clinical Signs

The age of the affected goats ranged from 2 to 5 years old (mean, 3.5 years old), and the flock had a history of 10 years of disease. Clinically, displayed clinical signs of ENA, including nasal discharge and noisy breathing, respiratory distress were the most prominent findings in the eleven affected animals, and also among most of the animals in the farm.

The mean values of serum telomerase value in ten healthy and 11 ENA-affected goat are shown in *Table 1*.

**Table 1.** Statistical analysis results of serum telomerase activity in infected and control groups

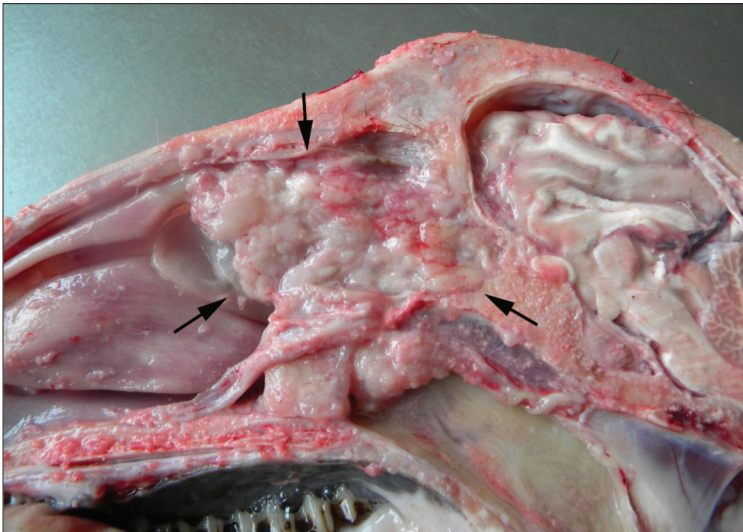
Control Group (ng/mL)	Infected Group (ng/mL)	P Value
18.97±5.58	49.52±5.39	<0.001

### Necropsy Findings

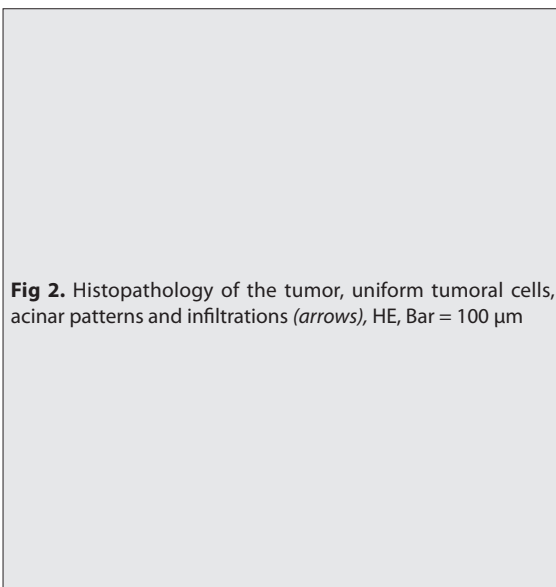
At necropsy, unilateral intranasal tumors were found in nasal cavity of all animals (*Fig. 1*). Distortion and blockage of the nasal canal were the common findings. Tumor tissues generally were soft but has hard areas in cut surface of the some tumoral. Seromucous exudate covered the tumor tissues in most of the cases. Pinkish white masses include hemorrhagic areas in rare cases. Although no metastasis was observed any cases, invasion to the nasal sinuses were seen in two cases.

### Histopathological Findings

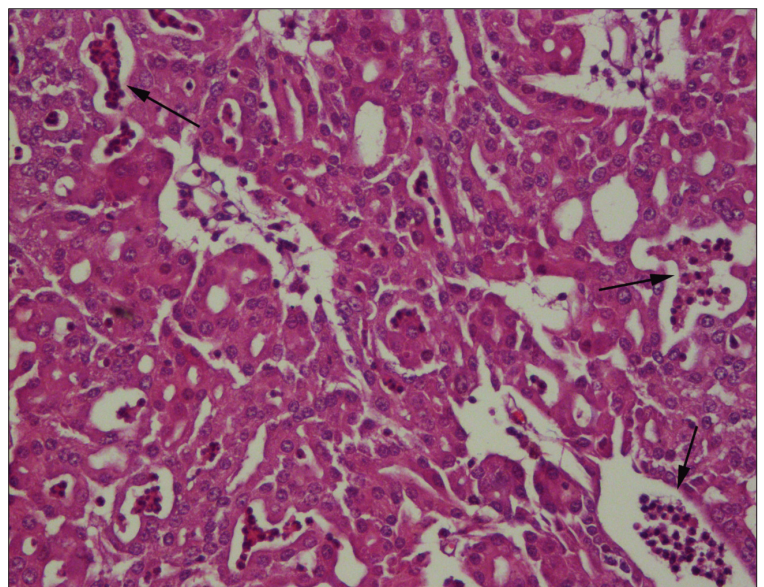
Histopathological examination revealed acinar, tubular and papillary patterns. Tumors contained well-differentiated, epithelial cells and scant fibrovascular stroma. The tumoral cells were in cuboidal to columnar shapes with distinct cell borders and eosinophilic cytoplasm. The nuclei were generally round or oval and centrally located. No indication

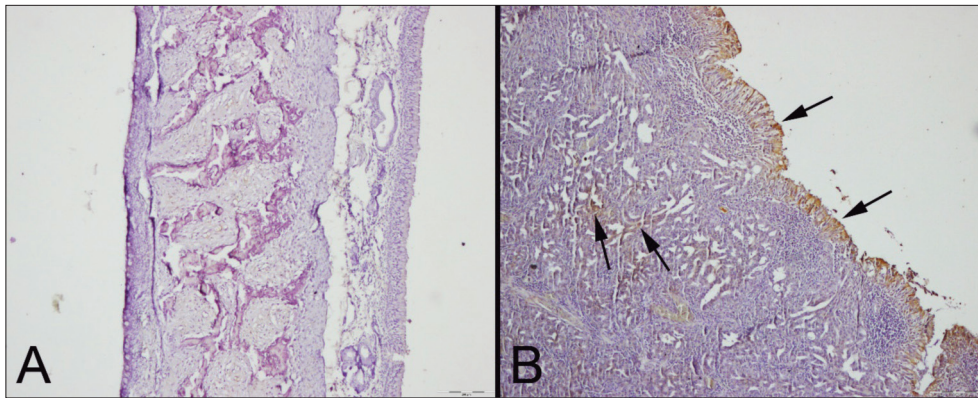


**Fig 1.** Gross appearance of ENA (arrows) localized in nasal cavity

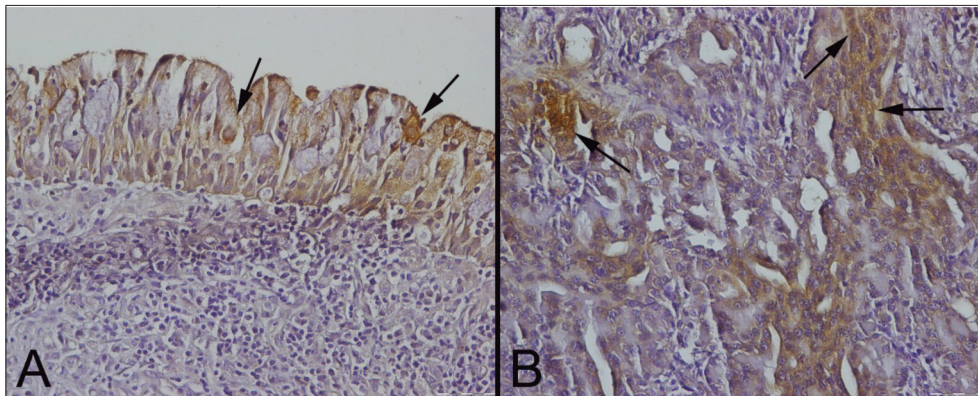


**Fig 2.** Histopathology of the tumor, uniform tumoral cells, acinar patterns and infiltrations (arrows), HE, Bar = 100 µm





**Fig 3.** A- Negative telomerase activity of the control nasal tissue, B- Marked increase of the telomerase immunoreaction (arrows) in tumoral mass, Streptavidin biotin peroxidase method, Bar = 100  $\mu$ m



**Fig 4.** A- Higher magnification of increased telomerase immunoreaction (arrows) in nasal epithelial cells, B- Increased telomerase immunoreaction (arrows) in tumoral cells. Streptavidin biotin peroxidase method, Bar = 50  $\mu$ m

of pleomorphism and cellular atypia were observed but, slight necrosis, inflammatory infiltrations and mitoses were seen (Fig. 2).

At the immunohistochemistry, increase in telomerase activity in the nucleus and cytoplasm of the tumoral cells was observed in granular appearance (Fig. 3, 4). The severity of the telomerase expression was not uniform and changing slight to severe in the tumoral mass.

## DISCUSSION

Enzootic nasal adenocarcinoma is a contagious neoplasm of the secretory epithelial cells of the nasal mucosa of small ruminants and the causative agents of the tumor is retrovirus. ENA is commonly spread horizontally, most likely by the respiratory route. Although clinical symptoms and chronic behavior suspected the diseases, the easiest way for to diagnosis is the examination of nasal cavity of dead animals. Histopathological and/or ultrastructural examinations are necessary for definitive diagnosis [25]. In this study diagnosis of ENA was made base on the clinical, macroscopically, microscopically and ultrastructural findings. Only weld differentiated and characteristic cases included this study.

The telomerase activity plays an important role in maintaining chromosome stability, cellular immortality and oncogenesis. Telomerase is highly active in 90% of human cancers and transformed cells possess longer telomeres at their chromosomes [26]. Beyond its role in telomere maintenance, telomerase provides additional functions in tumorigenesis, DNA repair and cell survival. Telomerase protects cells from apoptosis and necrosis, and stimulates growth in adverse conditions [27].

The telomerase enzyme is ribonucleoprotein reverse transcriptase enzyme that adds telomeric repeats into end of chromosomes [28]. This activity protects the integrity of chromosomes from digestion by exonucleases, fusion to the neighboring chromosomes and occurring the chromosomal defects [29,30]. Possible relation between carcinogenesis and telomere dysfunctions was reported [31,32]. Previous studies cataloging TERT expression and telomerase activity reported potent suppression in human somatic tissues, while robust expression and activity in germ cells and cancer cells. Progressive telomere shortening from cell division provides a barrier for tumor progression. However, one of the hallmarks of advanced malignancies is continuous cell growth and this almost

universally correlates with the reactivation of telomerase<sup>[32]</sup>. Many authors suggested that determination of telomerase activity may serve as a diagnostic and prognostic tool in oncology. The same results were also observed in tumor cells in animals<sup>[33,34]</sup>. Telomerase activity was also determined in malignancies of feline tissues and in canine mammary tumors. Results showed that measurement of telomerase activity may be an effective method for detecting malignancy in animals<sup>[34,35]</sup>. Because it is a viral and tumoral disease, in this study telomerase activity of ENA examined by immunohistochemically method in tissue and by ELISA in serum. Marked increases in serum and tissue telomerase activity observed in both serum and tissue.

Recent studies reported that telomerase activation has an important role in normal somatic cells, and that failure to activate sufficient telomerase also promotes disease. Similarly, some viruses modulate the telomerase activity in animal cells<sup>[36,37]</sup>. Result of the present study showed that telomerase activity is effected by the disease. This results also indicated that telomerase activity has an important role in ENA. Detection of the telomerase activity in tissue by immunohistochemical methods is recent and reliable method in human tumors. Telomerase expressed in cytoplasm and nucleus of the tumoral cells<sup>[38-40]</sup>. In this study we used this technique in ENA and our results showed that telomerase expression in tumor tissue was increased compared to normal nasal tissue. The expression was observed both intracytoplasmic and intranuclear localisations.

Effect of virus on telomerase activity in animals previously was reported<sup>[36,37]</sup> but there is no report about ENA virus and telomerase activity in goats. This study showed that ENA virus up-regulate telomerase activity of infected cells and serum level. Understanding the mechanisms by which ENA virus exerts this further studies are needed.

In recent times, the incidence of ENA has dramatically increased throughout the world. Thus, the prediction of tumor development may require a decrease in the economic damage incurred. Our results showed that telomerase activity becomes a useful prediction marker for tumor development in infected but clinically healthy goats in infected flocks. In this study we have shown that telomerase activity increase in serum and tumoral tissue in ENA cases in goats.

## CONFLICTS OF INTEREST

The authors declare no conflict of interest

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