The Continuum of Eustachian Tube Obstruction in Cats: A temporal bone study

Nevra KESKİN-YILMAZ1,2,a, Patricia A SCHACHERN2,b, İrem GÜL SANCAK2,3,c, Michael M PAPARELLA2,d Sebahattin CÜREOĞLU2,e

1Ankara University, Faculty of Veterinary Medicine, Department of Internal Medicine, Ankara, Türkiye; 2University of Minnesota, Medical School, Department of Otolaryngology, Head and Neck Surgery, Minneapolis, MN, USA; 3Ankara University, Faculty of Veterinary Medicine, Department of Surgery, Ankara, Türkiye

aORCID: 0000-0002-6287-1157; bORCID: 0000-0002-8427-8549; cORCID: 0000-0002-9108-8954; dORCID: 0000-0002-2727-1674; eORCID: 0000-0001-9220-7073

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Abstract

The Eustachian tube is a canal from the tympanic cavity to the nasopharynx that is responsible for the aeration, drainage, and pressure equalization of the middle ear. Partial or complete blockage of the tube can trigger severe ear disease. We evaluated pathologic changes from Eustachian tube obstruction (ETO) in 15 temporal bones from cats with bilateral ETO from the temporal bone collection at the University of Minnesota Medical School Otopathology Laboratory. The samples were evaluated for histopathological changes to investigate the continuum of the disease at intervals of 2 days, 1-week, 2-weeks and 4-weeks. Temporal bones were sectioned in the horizontal plane and every 10th section was stained with hematoxylin and eosin. One section from each ear was stained with periodic acid-Schiff and Alcian blue. Sections were studied under light microscopy. The results revealed moderate hyperplasia equally developed throughout the epithelial layer surrounding the middle ear and neutrophil-rich inflammatory cell infiltration. As the duration of obstruction prolonged to the 4th week, compositional change of the middle ear effusion from serous to mucoid that was accompanied with granulation tissue formation was observed. In conclusion, the severity of the findings related to ETO are directly proportional to the duration of the disease. Therefore, patients presenting with long-lasting complaints of ear diseases should be examined for dysfunction or blockage of the Eustachian tube.

Introduction

The Eustachian tube connects the tympanic cavity to the nasopharynx and functions in the ventilation, drainage, and pressure equalization of the middle ear (14, 20). Failure of the Eustachian tube to perform these functions defines Eustachian tube dysfunction. Since the functions of the Eustachian tube are quite complex and multifactorial, the etiology of Eustachian tube dysfunction is not fully understood (14, 20).

Previous studies (1, 6, 14), have identified a link between viral upper respiratory infections leading to Eustachian tube dysfunction, accompanied by an increased nasal inflammatory response. In later studies, allergen induced dysfunction of the Eustachian tube presenting with inflammation and edema of the middle ear epithelium has been described (1, 9, 10, 21). In addition to allergen stimuli, benign or malignant neoplastic formations originating from the middle ear and eustachian tube can also cause complete or partial obstruction of the Eustachian tube leading to nasopharyngeal signs and otitis (5, 8, 11). In Eustachian tube obstruction (ETO) or blockage, negative pressure increases and triggers the transition of plasma from mucosal vessels to the middle ear cavity, and undrained accumulated fluid results in serous otitis media (2, 4, 14). In this study, we describe the histopathologic changes in the middle and inner ears of cats from 2 days to 4 weeks after ETO.
Materials and Methods

Our temporal bone samples consisted of 15 cats with bilateral ETO from the animal temporal bone collection at the Otopathology Laboratory, University of Minnesota. The study was performed and the samples were added to the collection in the 1970s and at that time the temporal bone study was approved by the institutional review board.

In order to histopathologically identify the changes caused by ETO in the middle ear, 15 healthy cats were purchased from and housed individually by Research Animal Resources (RAR). Animals were given food and water ad libitum. All cats underwent detailed general examinations, including otoscopy, and cats that were determined to be healthy weighing 2 to 5.5 kg. They were pre-anesthetized with atropine (0.04 mg/kg) and continued with ketamine (10 mg/kg) and acepromazine (0.1 mg/kg). Before the procedure butorphanol (0.5 mg/kg) was injected subcutaneously for analgesia and oxytetracycline ophthalmic ointment was used for each eye. A midline incision was made in the soft palates to expose the orifices of the Eustachian tubes. Multiple pieces of silastic sponge were pushed into the Eustachian tubes bilaterally and the soft palates sutured with chromic catgut suture. Analgesic and antibiotic treatment (Penicillin G BID, 25000 U/kg) was continued for 3 days after surgery. The cats’ vitals and pain conditions were monitored every day. Pain management was established due to physiological and behavioral evaluation by trying to keep them content and quiet, comfortable when resting and interested about environmental surroundings. The cats were fed with soft food to be able to minimalize the pain on the surgery site.

To be able to follow the continuum of the histological changes, the animals were divided into 4 groups and euthanized at 2 days (n=3), and 1 (n=4), 2 (n=4), and 4 (n=4) weeks after obstruction. The temporal bones were removed and fixed in 10% formalin, dehydrated in a graded series of ethanol, decalcified with trichloroacetic acid, and embedded in celloidin. They were serially sectioned on the horizontal plane at a thickness of 20 μm. Every 10th section was stained with hematoxylin and eosin. Celloidin was removed from an additional section from each ear and stained with periodic acid-Schiff and Alcian blue as described previously (17). Sections were studied under light microscopy.

The mid modiolar section was used to measure the thickness of the middle ear mucosa at the inferior, anterior, and posterior wall using a digital camera and image analysis software (SPOT Advanced; SPOT Imaging Solutions, Sterling Heights, MI, USA). Three measurements were taken at each location and their values averaged.

Results

Two days after obstruction, there was very slight widening of the subepithelial layer of the middle ear. No other abnormalities of the middle ear structures were observed.

One week after obstruction, there was an increase in the thickness of the subepithelial layer compared to the 2-day obstructed group. Hyperplasia was equally developed throughout the epithelial layer surrounding the middle ear. Serous effusion filled the majority of the middle ear cleft. There was mild neutrophil-rich inflammatory cell infiltration mainly in the area around the round and oval window membranes.

At 2 weeks after obstruction, intense thickening, edema, hyper vascularization, and hyper dilatation of the vessels of the subepithelial layer of the middle ear were observed. Serous effusion filled the majority of the tympanic cavity while effusion with higher viscosity was observed around the Eustachian tube. There was neutrophil-rich inflammatory cell infiltration in other locations of the middle ear cavity. Additionally, neutrophil granulocyte and mononuclear cell infiltration were seen in the subepithelial space. The epithelium of the round window membrane that faces the tympanic cavity was covered with inflammatory cells. Goblet cells stained with Periodic acid Shiff and Alcian blue appeared magenta in color demonstrating the presence of neutral mucins.

After 4 weeks of obstruction, there was a transition of the middle ear effusion from serous to mucoid, with increased infiltration of plasma cells and other mononuclear cells. There was severe hyperplasia of the epithelial layer and hypervascularization of the subepithelial layer (Figure 1). Goblet cells that were predominantly in the mesotympanum and Eustachian tube stained dark blue to purple with periodic acid Shiff and Alcian blue indicating the presence of both neutral and acidic mucins (Figure 2). Some goblet cells had expelled their mucous contents and contained empty vacuoles. Mucoid effusion was mainly around the opening of the Eustachian tube in the middle ear cavity. Extensive granulation tissue was observed in the middle ear cavity (Figure 3). Although the small number of animals in each group did not permit statistical analysis of mucosal thickness, it did show a trend to increase overtime (Figure 4).
Figure 1. There is slight widening of the sub-epithelial layer of the middle ear after 2 days after Eustachian tube obstruction (a), that increases in weeks 1 (b), 2 (c), and 4 (d) Stained with haematoxylin and eosin. (ME=middle ear, hollow arrow: sub-epithelial layer of the middle ear). Scale bar: 100 µm.

Figure 2. a & b: After two weeks of obstruction goblet cells appear magenta in color indicating the presence of neutral mucins. There is increased activity of goblet cells. c & d: four weeks after obstruction periodic acid Shiff and Alcian blue staining that are dark blue to purple in color demonstrating continued goblet cells activity with a greater content of acidic mucins (ME= middle ear). Scale bar: 100 µm.
Discussion and Conclusion

Nasopharyngeal masses, cleft palate, neurologic and pulmonary diseases, and nasal and ocular discharge have been suggested to trigger Eustachian tube obstruction and serous otitis media (3, 7, 14, 19). In our study, we found that, Eustachian tube obstruction causes serous otitis media as early as 1 week post obstruction. We have also observed widening of the subepithelial layer in all temporal bone samples. Due to the small number of samples, it was not possible to do statistical analysis in this study. However, we have observed that the mucosa thickened with increasing periods of obstruction in the early periods from edema and at later periods from inflammatory infiltration. We have also revealed the hyperactivation of goblet cells, accumulation of exudate, capillary dilatation, and granulation tissue formation at later time intervals of obstruction. These findings are consistent with previous studies and associated with the inflammatory process (12, 13).

Previous studies have shown that the number and activity of goblet cells can increase in a very short time in association with the inflammatory or immune response (12, 16). In our study, we observed a dramatic increase in the activation of goblet cells in the middle ear mucosa in the first and second weeks. In the fourth week when the effusion transformed from sero-mucoid to mucoid, goblet cells were still active. Our histochemical evaluation using Alcian blue and periodic acid-Shiff staining showed neutral mucins at 2 weeks that by 4 weeks also contained acid mucins. It may be that the increase in acid mucins and viscosity are related.

We observed granulation tissue formation as early as 4 weeks after obstruction. Granulation tissue formation is often present in chronic otitis media (18). The phenomenon of granulation tissue formation was summarized in three steps: Rupture in the surface epithelium and accompanying cellular infiltration from the lamina propria to the middle ear cavity, expulsion through the ruptured region of the epithelial cell surface of the separated lamina propria and finally the beginning of re-epithelialization (18). Negative ear pressure causes a more severe decrease in sound transmission than positive...
pressure and leads to conductive hearing loss. When effusion and granulation tissue formation coexist in the middle ear space, there is a reduction in the compliance of the tympanic membrane and a further reduction in sound transmission (15).

Dysfunction of the Eustachian tube should be considered as a disease with many complications. In this study, epithelial hyperplasia and the composition of middle ear effusion are directly proportional to the duration of Eustachian tube obstruction and untreated cases may develop chronic ear infections with granulation tissue formation. Considering the major impact of Eustachian tube dysfunction on otitis media and related surgeries, the early symptoms should be carefully addressed and followed-up.

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Conflict of Interest
The authors have no financial relationships, or conflicts of interest to disclose.

Author Contributions
NKY, PAS, MMP and SC conceived and planned the study, NKY, PAS, MMP carried out the experiments and collected the data, NKY, PAS, IGS performed the data analysis, NKY, PAS and SC took the lead in writing the paper. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Data Availability Statement
The data supporting this study’s findings are available from the corresponding author upon reasonable request.

Ethical Statement
This study was approved by the institutional review board of the University of Minnesota (No:#00003249).

Animal Welfare
The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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