Trypsin Identified in Middle Ear Fluid of Children with Serous Otitis Media

Omer N. Develioglu1, Halim A. Is1, Hakan Ekmekci2, Ozlem Ekmekci2, Zeynep Gungor1, Murat Yener1, Mehmet Yilmaz1, Gunay Can4, Mehmet Kulekci1, Ozgun Enver3, Huseyin Sonmez2, and Mine Kucur2*

1Department of Otorhinolaryngology, Head&Neck Surgery, Taksim Education and Research Hospital, Turkey
2Department of Medical Biochemistry, Istanbul University, Turkey
3Department of Otorhinolaryngology, Head&Neck Surgery, Istanbul University, Turkey
4Department of Public Health, Istanbul University, Istanbul

Abstract

Objectives: Gastroduodenal agents may have the differential role in GERD (gastroesophageal reflux disease). Besides pepsin and bile acid reflux, trypsin might also play a significant role in the middle ear pathologies. The aim of the current study was to identify trypsin with the presence of bile acid and pepsin in the middle ear fluids of children undergoing myringotomy and tube placement for OME, to compare it with the serum levels, and to investigate possible relationship between GER and OME pathogenesis.

Study design: Randomized clinical trial.

Setting: Tertiary health center.

Subjects and methods: Twenty-five patients (14 boys, 11 girls) with OME who underwent myringotomy with tube placement at the Department of Otorhinolaryngology of Taksim Education and Research Hospital and Cerrahpasa Medical Faculty Otorhinolaryngology and Head&Neck Surgery were enrolled in a prospective study to evaluate the presence of pepsin, bile acid and trypsin in middle ear aspirates. Cochlear implant group (n:25) was considered as the control group.

Results: Measurable levels of pepsin, bile acid and trypsin were present in all MEEFs from the patients. The difference between the levels of pepsin, bile acid and trypsin measured in MEE and serum was statistically significant (p=0.001).

Conclusion: We were able to demonstrate for the first time the presence of trypsin in middle ear samples of children with OME. Reflux of the duodenal contents including trypsin has been reported to contribute to the development of esophageal mucosal damage and inflammation. By similar mechanisms trypsin might play a role in the development of otitis media.

INTRODUCTION

Chronic otitis media with effusion (OME) is characterized by the presence of a middle ear effusion (MEE) in the middle ear space for 3 months or longer [1]. The pathogenesis is thought to be multifactorial. Viruses, bacteria, dysfunction of the Eustachian tube, anatomical factors, allergy, familial predisposition, male sex, wrong methods of feeding, and environmental factors have been suggested to contribute to this chronic inflammation process [2].

Recently gastroesophageal reflux (GER) has gained considerable interest for its potential role in the etiopathogenesis of OME. GER, a common and often a physiologic occurrence in infants and children, decreases with age [3].

In the study by Tasker et al. [4], pepsin levels in the effusion samples were measured up to a 1000 times higher than the serum levels. Further proof of the involvement of GER in children with OME was also supported by Tasker et al. They found bile acids in 32% of children with OME. Lieu et al. [5], measured pepsin levels in MEE of children; 73% of the patients were positive with proteolytic assay. They speculated that gastric reflux must certainly be the source rather than plasma transudate or local production by the middle ear.

Gastroduodenal agents may have the differential role in GERD (gastroesophageal reflux disease). Besides pepsin and bile acid reflux, trypsin might also play an important part in the middle ear pathologies. It has been evaluated that trypsin presence in the refluxate with bile acids has been associated with more extensive esophageal mucosal damage instead of pepsin.
Recurrent otitis media cases show evidence of chronic inflammation process. Although there have been various studies establishing a causal relation between GER (pepsin and bile acid) and otitis media [6-9], the presence of trypsin in the middle ear effusion have not been yet elucidated.

The aim of the current study was to identify trypsin with the presence of bile acid and pepsin in the middle ear fluids of children undergoing myringotomy and tube placement for OME, to compare it with the serum levels, and to investigate possible relationship between GER and OME pathogenesis.

**MATERIALS AND METHODS**

**Study subjects**

Twenty-five patients (14 boys, 11 girls) with OME who underwent myringotomy with tube placement at the Department of Otorhinolaryngology of Taksim Education and Research Hospital and Cerrahpasa Medical Faculty Otorhinolaryngology and Head&Neck Surgery were enrolled in a prospective study to evaluate the presence of pepsin, bile acid and trypsin in middle ear aspirates. Cochlear implant group (n:25) was considered as the control group. Demographic and clinical data including patient age, sex, medical history, admitting diagnosis and operative procedure were recorded. Patients’ ages ranged between 4 and 13 years. They had a clinical diagnosis of OME (the presence of MEE for 3 months or longer) and appropriate criteria for placement of a ventilation tube.

Samples were measured with an enzyme-linked immunosorbent assay (ELISA) using human pepsin-, human total bile acid- and human trypsin-specific antibodies (Hangzhou Eastbiopharm Co, Ltd, Yale Road, Hangzhou). The study was approved by the Institutional Review Board of Cerrahpasa Medical Faculty and informed consents were obtained from the parents of the patients.

**Criteria for Diagnosis of Otitis Media**

Patients were scheduled for bilateral myringotomy with tubes based upon clinical history and otoscopic evaluation in a tertiary care pediatric otolaryngology clinic using standard accepted criteria of the American Academy of Family Physicians, American Academy of Otolaryngology-Head and Neck Surgery, and American Academy of Pediatrics Subcommittee on Otitis Media with Effusion.

**Exclusion criteria**

Exclusion criteria included diagnosis of craniofacial disorder or known syndrome and diagnosis of other chronic illness.

**Middle ear fluid sampling**

Myringotomy with tube insertion was performed under general inhalation anesthesia in the operating room. At the time of myringotomy (prior to placement of the tube), a suction cannula was placed through the myringotomy incision into the middle ear cavity. If fluid was present, this was aspirated entirely into Juhn Tym-Tap (Medtronic Xomed, Jacksonville); then while the cannula was maintained in the middle ear space, 1 mL of sterile saline was flushed into the external auditory canal and allowed to flow into the middle ear and then aspirated with the suction cannula. This was to enable tenacious secretions to be flushed from the suction cannula into the specimen container for analysis. If no effusion was present in the middle ear, to recover any pepsin, bile acid or trypsin adherent to the middle ear mucosa, the cannula was placed through the myringotomy incision and 1 mL of sterile saline was flushed into the external canal and allowed to bathe the middle ear cavity prior to aspiration. The fluid aspirated into Juhn Tym Tap was immediately transferred to a freezer and stored -80°C in preparation for assay. Ears were categorized by the operating surgeons at the time of surgery as being “dry” or “with effusion” based on the otomicroscopic appearance of the middle ear after myringotomy and recorded in the operative note.

In the laboratory, the middle ear fluid was collected in a polystyrene tube and centrifugated for 10 minutes at 2500 rpm.

**Sampling of the middle ear in the cochlear implant group**

Middle ear fluid from the cochlear implant group is used as a control group. The cochlear implant surgical approach involved a transmastoid transfacial recess exposure of the middle ear to allow for cochleostomy and the electrode array insertion. Patients undergoing unilateral or bilateral cochlear implantation were included. The mastoid and middle ear dect of all patients undergoing implantation were free of effusions at the time of surgery. Care was taken to minimize the amount of irrigation used during drilling. Irrigation fluid was not used once the antrum had been identified and opened widely enough to visualize the aditus ad antrum to ensure that the irrigation did not wash out the middle ear cleft. 1 mL of saline was instilled via an angiocatheter into the middle ear dect through the aditus ad antrum and aspirated into Juhn Tym Tap after it had bathed the middle ear space. The fluid aspirated into Juhn Tym Tap was immediately transferred to a freezer and stored -80°C in preparation for assay.

**Blood collection**

Blood samples were obtained from each child together with the middle ear fluid. All samples were stored at -80°C until they were processed.

**Data analysis**

Pepsin, bile acid and trypsin concentrations were expressed as mean with SD. Data were analysed to look for significant differences between patient and control groups using the Student t test and the Fisher’s exact test. P value <0.05 was considered a statistically significant difference.

**RESULTS**

A total of 50 subjects (25 patients and 25 controls) undergoing myringotomy with tube placement and cochlear implantation were included in this study. The mean age was 8.02 ± 2.37 ranging between 4 to 13 years? A total of 100 samples were tested with ELISA specific for human pepsin, bile acid and trypsin. Both middle ear fluid levels and serum levels of pepsin, bile acid and trypsin were measured. Measurable levels of pepsin, bile acid and trypsin were present in all MEEEs from
the patients. The difference between the levels of pepsin, bile acid and trypsin measured in MEE and serum was statistically significant (p=0.001) (Table 1, Figure 1).

**DISCUSSION**

This is the first study to identify trypsin with the presence of bile acid and pepsin in middle ear effusion samples of children with OME. We detected measurable levels of trypsin, bile acid, pepsin in all effusion samples using a commercial enzyme-linked immunosassay human kit.

Reflux of the duodenal contents with gastric acid has been reported to contribute to the development of esophageal mucosal damage [10-12]. Trypsin, one of the proteases in the pancreatic juice has in particular been reported to play a significant role in the development of esophageal mucosal injury. Previous studies have demonstrated that trypsin has a synergistic effect on the development of mucosal injury with bile acid, though trypsin alone does not induce esophageal mucosal injury [13]. Although it has been considered that bile salts under acidic and alkaline conditions do not cause morphologic injury, Develioglu et al. [14], recently reported that the presence of bile acids in middle ear effusions associated with more extensive mucosal damage than pepsin. The middle ear effusion pH ranges from 6.0 to 9.0 [4,10].

Pepsin becomes active at a pH below 4.5 and has its optimum activity at pH 2.0. Therefore, pepsin may have minimal activity in the middle ear and can be inhibited irreversibly by the high pH of the effusion [12]. Bile acids are already noxious at a pH range between 3 and 6 and possibly even at neutral pH. These qualities would make bile acids potentially more harmful agent to the middle ear mucosa than pepsin.

The precise damaging mechanisms of the esophageal mucosa by trypsin have been investigated before, but it is unclear if similar mechanisms apply for the middle ear mucosa. Recent studies show that pancreatic trypsin can stimulate the production of inflammatory mediators, including chemokines and prostaglandins from human esophageal epithelial cells in vitro [15]. In the present study we found high concentrations of trypsin with the presence of bile acid and pepsin in middle ear samples of children with OME. It has been well known that increased middle ear effusion was generally coupled with inflammation of middle ear mucosa. By means of these properties, we can assume that trypsin may play a significant role in the development of middle ear mucosal inflammation.

In recent studies, it has been shown that trypsin induces the expression of proinflammatory cytokines in epithelial cells

| Table 1: Evaluation of pepsin, bile acid and trypsin levels, mean ± SD. |
|-----------------|-----------------|-----------------|------------------------------|
|                 | Cochlear implant | OME             |                             |
|                 | Mean ±SD        | Mean ±SD        | P                            |
| MEE             |                 |                 |                              |
| Pepsin          | 7,55 ± 3,13     | 29,56 ± 12,35   | .001***                      |
| Bile acid       | 8,29 ± 7,25     | 33,47 ± 9,15    | .001***                      |
| Trypsin         | 63,59 ± 20,58   | 107,63 ± 20,49  | .001***                      |
| Serum           |                 |                 |                              |
| Pepsin          | 67,96 ± 15,12   | 127,79 ± 38,75  | .001***                      |
| Bile acid       | 9,28 ± 6,48     | 60,03 ± 18,90   | .001***                      |
| Trypsin         | 198,52 ± 87,87  | 549,93 ± 218,48 | .001***                      |

*p=0.001 compared with cochlear implant as control group.

![Figure 1](image) Pepsin, bile acid and trypsin levels in MEE and serum.
through the activation of specific receptors, protease-activated receptors (PARs). These data might suggest that trypsin might play a crucial role in middle ear inflammation induced by gastroduodenal reflux.

Epithelial paracellular passage of potentially noxious agents plays an important pathophysiological role in the initiation of GER [16]. Bjorkman et al., reported that GERD patients had increased epithelial permeability. Duodenal reflux components including bile salts and trypsin, have the potential to disrupt the esophageal barrier function. Shan et al demonstrated that trypsin impaired epithelial barrier function through the activation of the PAR-2. Impaired epithelial barrier property might increase the intensity of the noxious effect of these components despite protective mechanisms [17]. The middle ear is a kind of cul-de-sac and this anatomy may result in a longer contact with harmful duodenal contents that might lead to extensive mucosal damage induced by trypsin by similar mechanisms. This might play a role in the development of OM.

The present study differed from the aforementioned reports in that we focused the pancreatic trypsin exposure rather than regular reflux. This study is the first to identify trypsin in the middle ear effusion and supports the assumption that pancreatic trypsin is involved in middle ear mucosal inflammation caused by gastroduodenal reflux. One of the limitations of our study was that we did not investigate the underlying mechanisms of noxious effects of trypsin. Further studies may be designed to indicate probable trypsin/PAR-2 activation IL-8 production in middle ear mucosa to support our findings.

CONCLUSION

We were able to demonstrate for the first time the presence of trypsin in middle ear samples of children with OME. Reflux of the duodenal contents including trypsin has been reported to contribute to the development of esophageal mucosal damage and inflammation. By similar mechanisms trypsin might play a role in the development of otitis media.

DECLARATION OF INTEREST

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