Detection of gastric pepsin in middle ear fluid of children with chronic otitis media with effusion

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Objectives
To study the possible relationship between gastroesophageal reflux and chronic middle ear effusion by (i) evaluating the presence of pepsin/pepsinogen in middle ear fluid of children with chronic otitis media with effusion (OME) and to (ii) differentiate between pepsin from gastric juice and pepsin derived from plasma pepsinogen.

Patients and methods
This was a prospective study carried out in the Departments of Otorhinolaryngology and Clinical Pathology, Alexandria University. Middle ear effusions and blood samples were collected from 25 patients undergoing bilateral myringotomy with tube placement for chronic OME. These samples were prepared for pepsin assay. The total pepsin/pepsinogen concentrations of effusions and serum samples were measured with an enzyme-linked immunosorbent assay using human pepsin-specific antibody.

Results
Pepsin was detected in 22 of 25 (88%) patients and in 42 of 50 (84%) of the ears, and it was detected in all serum samples. A total of seven of 25 (28%) patients and nine of 50 (18%) ears were found to have higher pepsin levels in their middle ear effusion samples than the cut-off value of serum. The average pepsin level in all effusion samples was 109.99 ng/ml, whereas it was 55.72 ng/ml in serum samples.

Conclusion
The gastroesophageal reflux is one of the contributing factors in the etiopathogenesis of middle ear effusion as gastric pepsin reaches the middle ear through the nasopharynx and Eustachian tube to cause OME.

Keywords:
gastroesophageal reflux, otits media with effusion, pepsin

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Introduction

Otitis media with effusion (OME) is characterized by the collection of serous or mucous fluid behind an intact tympanic membrane during an inflammatory process and the lack of acute signs and symptoms of infection. It leads to a reduction in the tympanic membrane mobility and conductive hearing loss. When inadequately treated or left untreated, it may lead to sequelae and complications such as retraction pockets, tympanosclerosis, adhesive otitis media, permanent hearing loss, and impairment in development of speech and language [1].

Multiple risk factors for OME in children have been identified, including adenoid hypertrophy, bacteria, smoke exposure, presence of allergy, order of birth, craniofacial anomalies, ethnicity, age, season of the year, lack of breastfeeding, and genetic predisposition. However, the role of gastroesophageal reflux (GER) or extraesophageal reflux in this disease has only been recognized recently [2].

Once these reflux events produce pathological changes in the esophagus or upper aerodigestive tract, they are termed GER disease or extraesophageal reflux disease [3,4].

Extraesophageal reflux disease has been implicated in various pathological conditions of the upper aerodigestive tract in children including aspiration pneumonia, chronic cough, recurrent bronchitis, reactive airway disease, recurrent croup, subglottic stenosis, chronic laryngitis, sinusitis, adenotonsillar hypertrophy, otitis media, and otalgia [5].

Reflux of gastric contents into the nasopharynx and then into the middle ear cleft has been reported as a contributing factor in the development of OME [6,7]. The repeated exposure of the ciliated respiratory epithelium to pH 4 or less blocks ciliary movement and mucus clearance. HCl and pepsin cause local inflammation, edema, and ulceration of the respiratory mucosa, leading to loss of tube ventilatory function. This leads to impaired pressure equalization with persistent negative pressure, a condition that has been shown to result in middle ear effusions [8,9].

Middle ear effusions partly consist of a plasma transudate. Thus, it is important to detect the origin of the pepsin of middle ear effusion and wether it is derived from gastric juice or from plasma transudate.

The aim of this work is to study the possible relationship between GER and chronic middle ear effusion by evaluating the presence of pepsin/pepsinogen in middle ear fluid of children with chronic OME and compare it with its serum concentration.

Patients and methods

This was a prospective study carried out between January 2012 and July 2012 in the Departments of Otorhinolaryngology and Clinical Pathology, Alexandria University, on 25 children with chronic OME that was refractory to the usual lines of conservative treatment for at least 3 months.

Institutional review board approval was obtained for this research and informed consents were obtained from the parents of the patients.

Patients were scheduled for bilateral myringotomy with tube placement on the basis of the history, otoscopic evaluation, and presence of type-B tympanogram.

Children with a history of allergic disease, cleft palate, Down syndrome, and current use of an H_2 blocker or a proton pump inhibitor were excluded.

All participants in this study underwent bilateral myringotomy with tube placement. Middle ear effusions and blood samples were collected and prepared for pepsin assay.

The effusion fluid samples were centrifuged to separate cellular components, and the supernatant was stored at −80°C for later pepsin assay.

The blood samples were allowed to clot for 30 min at room temperature and centrifuged for 5 min to separate the serum. The separated serum was stored at −80°C. Of the examined samples, the hemolytic ones were all excluded as sample hemolysis would influence the result.

The total pepsin/pepsinogen concentrations of effusions and serum samples were measured with an enzyme-linked immunosorbent assay (ELISA) using human pepsin-specific antibody. The pepsin antibody recognizes both pepsin and pepsinogen protein. The kit is a competitive inhibition enzyme immunoassay for the in-vitro quantitative measurement of human pepsin in serum, plasma, tissue extraction samples, and other biological fluids.

All samples were brought to room temperature slowly and diluted according to the prediction of the pepsin/pepsinogen concentration in ear effusion and serum before assaying. If the pepsin concentration values for these samples were not within the range of the standard curve, further dilution was performed to obtain the proper result. Fresh samples without long-time storage are recommended for the test to prevent protein degradation and denaturalization, which would finally lead to erroneous results. Samples were then loaded into ELISA kit wells and instructions were followed as per the kits' protocol.

This assay has high sensitivity and excellent specificity for the detection of human pepsin. The minimum detectable dose of human pepsin is typically less than 0.47 ng/ml.

The normal serum pepsin level is 49.8–86.6 ng/ml. All pepsin values above 90 ng/ml were considered statistically significant for presence of gastric pepsin rather than transudation from plasma.

Results

Data were analyzed to examine significant differences between sample groups using the Mann–Whitney *U*-test and Fisher's exact test. A *P* value more than 0.05 was considered a statistically significant difference and a *P* value of 0.001 or less was considered a highly statistically significant difference.

Of the 25 patients in this study, 16 (64%) were males and nine (36%) were females. The mean age of the patients was 5.08 ± 2.43 years, with a range of 1–11 years.

Detection of pepsin in ear effusion and serum samples

Of 25 patients who underwent bilateral myringotomy with tube placement, we obtained 50 middle ear effusion samples and 25 serum samples. Using ELISA, the presence of pepsin was tested in all effusion and serum samples (Fig. 1).

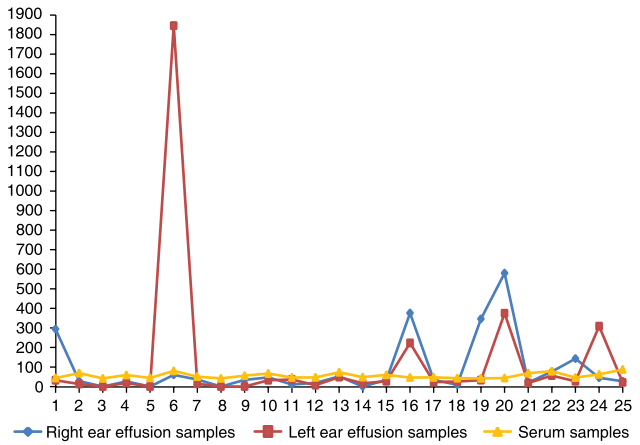
Pepsin was detected in 22 of 25 (88%) patients and in 42 of 50 (84%) of the ears and it was detected in all serum samples.

Source of pepsin in effusion samples

The average pepsin level in all effusion samples was 109.99 ± 282.27 ng/ml (range 0–1846 ng/ml), whereas it was 55.72 ± 13.77 ng/ml (range 42–87 ng/ml) in serum samples.

A total of seven out of 25 (28%) patients and nine out of 50 (18%) ears were found to have higher pepsin levels in

Figure 1



Level of pepsin in all effusion and serum samples.

their middle ear effusion samples than the cut-off value of 90 ng/ml and considered significant for presence of gastric pepsin rather than transudation from plasma. The average pepsin level of these effusion samples was 499.06 ± 14.05 ng/ml (range 143–1846 ng/ml), which was 8.98 times higher than those found in serum.

There was a statistically significant difference ($P = 0.001$) between the average pepsin level in effusion samples (with pepsin level >90 ng/ml) compared with the pepsin level in the serum.

Effect of age on the prevalence of high pepsin level in the effusion samples

The 25 patients were stratified into two groups by age: 5 years or younger and more than 5 years. Higher pepsin levels of above 90 ng/ml were detected in six of 16 (37.5%) children in the younger age group (≤5 years), whereas it was detected in one of nine (11.11%) children in the older age group (>5 years).

The younger group (≤5 years) had a significantly higher pepsin level in the effusion samples.

Discussion

OME is the most common cause of deafness in children; it is vital to elucidate its pathophysiology to allow for more effective prevention and treatment.

The etiology of OME is largely considered to be multifactorial, including factors such as infections, allergies, Eustachian tube dysfunction, adenoid hypertrophy, etc.

Recently, there has been an increasing interest in GER disease as one of the major contributing factors to this condition [6,10–13].

White *et al.* [14] reported that Eustachian tube function is altered in animals exposed to reflux. Butehorn *et al.* [15] showed that tubal opening was impaired after a transtympanic injection of HCl and pepsin. Heavner *et al.* [9] reported progressive impairment of the ventilatory function and mucociliary clearance with repeated transtympanic injections of HCl and pepsin.

The likely mechanisms for the existence of pepsin in otitis media with effusion include:

(1) Endogenous production of pepsinogen in the middle ear;
(2) GER;
(3) Transudation from serum.

However, pepsinogen mRNA was not detected in human middle ear mucosa [16].

In our study, pepsin was detected in 88% of all patients and 84% (42/50) of all ears. All serum samples were positive for pepsin assay.

A total of seven of 25 (28%) patients and nine of 50 (18%) ears were found to have higher pepsin levels in their middle ear effusion samples than 90 ng/ml and considered positive for the presence of reflux-induced OME. The average pepsin level of these effusion samples was 499.06 ± 14.05 ng/ml (range 143–1846 ng/ml), which was 8.98 times higher than those found in serum.

Tasker *et al.* [12] harvested middle ear effusions from 54 children. They measured pepsin concentrations using ELISA and enzyme activity assays. They reported that 83% (45/54) of the patient samples contained pepsin/pepsinogen at concentrations of up to 1000 fold greater than pepsin concentrations in the serum [12].

Nair *et al.* [10], in a prospective trial study, found that about 65.63% (21/32) of patients with OME had higher pepsin levels in their middle ear effusion samples than the cut-off value of 90 ng/ml.

O'Reilly *et al.* [6], in their study of a large cohort of patients with OME, found that the incidence of pepsin of the effusion samples was 14% (125/893).

He *et al.* [13] studied a large cohort of patients with OME and found that 14.4% (22/152) of the patients had detectable pepsin activity in one or both of the ear samples, with no pepsin activity detected in control serum.

The incidence of detection of higher pepsin concentration (>90 ng/ml) in the younger age group (≤5 years) was 37.5% (6/16) compared with 11.11% (1/9) in the older age group (>5 years). Thus, there

was a statistically significant correlation between age and a high level of pepsin in the middle ear effusion.

O'Reilly *et al.* [6] studied 509 patients and they were stratified into three age groups. The rate of positive pepsin assay in each group was analyzed. The younger group (<1 year) had a significantly higher rate of positive pepsin assay as compared with the other (older) two age groups [6].

Conclusion

The GER is one of the contributing factors in the etiopathogenesis of middle ear effusion as gastric pepsin reaches the middle ear through the nasopharynx and the Eustachian tube to cause OME, and therefore, control of GER may play a role in the prophylaxis and management of OME and avoidance of tympanostomy.

Acknowledgements

Conflicts of interest

None declared.

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