



## Diagnosis of extraesophageal reflux in children with chronic otitis media with effusion using Peptest



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### ABSTRACT

**Introduction:** The aim of the study was to investigate whether Peptest, an immunoassay used to detect pepsin, could be used to diagnose extraesophageal reflux (EER) in children with chronic otitis media with effusion (OME). The results obtained using this fast, simple and non-expensive method were compared with the results of previous studies.

**Methods:** Children 1–7 years old who had been diagnosed with OME and who were undergoing myringotomy with insertion of a ventilation tube were included in the prospective study. Middle ear fluid obtained during myringotomy was analyzed with Peptest to determine the presence of pepsin, and hence EER.

**Results:** Bilateral and unilateral myringotomy was performed in 15/44 (34.1%) and 29/44 (65.9%) children, respectively. Pepsin in the middle ear was detected in 14/44 (31.8%) children and in 19/59 (32.2%) middle ear specimens. Serous and mucous samples were positive for pepsin in 11/32 (34.4%) and 6/27 (22.2%) cases, respectively. Pepsin in the middle ear was detected in 3/7 children (42.9%) with bronchial asthma ( $p = 0.662$ ).

**Conclusions:** Pepsin was detected in 1/3 of middle ear specimens of patients with OME. These patients probably suffer from more severe reflux and therefore would be potential candidates for antireflux therapy. However, this has to be confirmed in further studies.

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### 1. Introduction

Acute otitis media (AOM) and chronic otitis media with effusion (OME) are among the most frequent causes for visits to the doctor in children 1–3 years old, and remain major health and socioeconomic issues [1]. It is estimated that up to 60% of children have experienced at least one episode of AOM by age 7 [2,3]. There are several well-known conditions that cause or facilitate the development of middle ear infection. The most important are upper respiratory infections, allergies, and adenoids [4].

Extraesophageal reflux (EER) has recently been found to be an additional risk factor for AOM and OME [5–10]. The Eustachian tube is not fully developed in children; it is shorter and more horizontal compared to that of adults. Therefore, it is easier for

refluxed content to reach the middle ear [11]. Many studies have shown that contact between refluxed content and mucous tissue of the nasopharynx, Eustachian tube, or middle ear can cause local inflammation and edema and thus facilitate the development of middle ear inflammation [5–9,12,13].

EER can be diagnosed directly by pH monitoring (pharyngeal or oesophageal), and also indirectly by detection of pepsin in tissues and fluids (e.g. sputum, saliva, middle ear fluid) [14–16]. Detection of pepsin in fluids and tissues is considered by some authors to be perhaps more appropriate than pH monitoring, because it reflects the long-term effects of EER and proves that EER is truly affecting the examined region. This is particularly true for more distant regions like the middle ear [16]. There is no pepsin in the middle ear under normal physiological conditions [5]. The presence of pepsin in the middle ear is therefore considered indirect confirmation of previous episodes of reflux into the middle ear.

To date, pepsin in fluids and tissues has been detected using expensive and time-consuming methods (e.g., ELISA, Western blot, and enzyme assays) [5,6,8,9]. These are not ideal for routine diagnostic use. The aim of this study was to investigate whether Peptest, a fast, simple and non-expensive method used for the

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detection of pepsin, could be used to diagnose EER in children with OME. The results obtained were compared with the results of previous studies.

## 2. Materials and methods

This prospective case-series study was approved by the Ethics Committee of the University Hospital and was performed in accordance with the Declaration of Helsinki, good clinical practice, and applicable regulatory requirements. The study was registered on ClinicalTrials.gov under the identifier NCT02183974. Written informed consent was obtained from both parents before initiating any procedure.

Children 1–7 years old who had been diagnosed with bilateral or unilateral OME and who were undergoing myringotomy with insertion of a ventilation tube were included in the study. OME was defined as effusion in the middle ear behind an intact eardrum for >3 months. Diagnosis was made on the basis of otomicroscopic findings, pneumatic otoscopy, type B tympanometry, and audiometry (in cooperative older children). Children with craniofacial abnormalities (e.g., Down syndrome, Treacher Collins syndrome, and clefts) were excluded from the study. Demographic data (including tobacco exposure) and symptoms of EER disease were provided by parents, who were also specifically questioned regarding the presence of hoarseness, recurrent lower respiratory infection (e.g., bronchitis and pneumonia), and bronchial asthma in their child.

When enlarged adenoids were present, adenoidectomy was performed prior to insertion of the ventilation tube. Myringotomy under magnification was performed in the anterior inferior part of the tympanic membrane. The type of middle ear effusion (i.e., fluid or mucous) was noted. Middle ear fluid was collected with a Tympanocentesis Collector 1419020 (Medtronic, Minneapolis, MN, USA), and a ventilation tube was inserted into the tympanic membrane. In cases of bilateral OME, bilateral ventilation tube insertion was performed simultaneously and the effusion was collected and analyzed separately. Analyses were performed immediately after surgery. First, 0.1 ml of 10% citric acid was added to the specimen. Afterwards, the specimen was centrifuged at 4000 rpm for 5 min. A small bench centrifuge, which is easy to operate and can be used at clinic, was used. If a clear supernatant layer was not visible, the sample was centrifuged again. An 80  $\mu$ l sample was drawn from the clear supernatant layer, added to a screwtop microtube containing 240  $\mu$ l of migration buffer, and mixed with a vortexer for 10 s. Afterwards, the specimen was assayed with Peptest (RD Biomed Limited, Hull, UK), which contains monoclonal antibodies targeted to pepsin and therefore detects the presence of pepsin (though not its activity). The results were noted after 15 min. Peptest results were specified as positive (two lines), negative (one line), or invalid (no line). Statistical analysis was performed using Microsoft Excel. Fisher's exact test was used to ascertain whether pepsin was more often present in patients with bronchial asthma.

## 3. Results

A total of 44 children, 24 boys (54.5%) and 20 girls (45.5%), were included in the study, which took place between June 2012 and March 2014. The average age of the study group was 4.2 years. Bilateral myringotomy was performed in 15 (34.1%) children, and unilateral myringotomy was performed in 29 (65.9%) children. Seven (15.9%) children had bronchial asthma. None of the children was taking medication for gastroesophageal reflux disease.

Altogether, 59 middle ear fluid specimens were examined, 30 from children with bilateral OME and 29 from children with unilateral OME. Pepsin in the middle ear specimen was detected in

14 (31.8%) children. In five children with bilateral OME, pepsin was detected in the middle ear fluid in both ears. Moreover, pepsin was detected in the middle ear fluid of nine patients with unilateral OME. Thus, pepsin was detected in 19/59 (32.2%) middle ear specimens. No invalid results were noted. Serous samples were positive for pepsin in 11/32 (34.4%) cases, while mucous samples were positive in 6/27 (22.2%) cases. Pepsin in the middle ear was detected in 3/7 children (42.9%) with bronchial asthma. No significant difference was noted between children with and without bronchial asthma ( $p = 0.662$ ).

## 4. Discussion

EER has been recently identified as a possible risk factor for OME [5–10]. Although important, diagnosis of EER in patients with OME is not easy. The simplest means of collecting information about reflux problems is questioning potential sufferers [17,18]. However, although many questionnaires have been developed over the last few years, even for infants and small children, questioning is still not a suitable method for the evaluation of EER in children, the reason being that symptoms of EER are heterogeneous and very common. Moreover, questionnaires are usually filled out by parents, who could interpret their child's symptoms incorrectly [17,18].

Currently, 24-h dual-probe esophageal pH monitoring or impedance is considered the best diagnostic method for EER. There is evidence of a 10-fold higher risk of the development of recurrent AOM or OME in children in whom EER is detected by means of double-probe esophageal pH monitoring [10]. 24-h oropharyngeal pH monitoring is a newer method aimed at detecting episodes of reflux to the oropharynx, and seems to generate similar results [14,19]. However, these methods may not be tolerated well, especially by children 2–7 years old. Moreover, the position of the sensor, which is placed in the hypopharynx or oropharynx, does not reflect the amount of reflux content that reaches the middle ear. Another disadvantage of pH monitoring is that it enables only short-term analysis over a timespan of just 24–48 h.

The measurement of pepsin in middle ear effusions would appear to be the most suitable method for evaluating a possible relationship between middle ear inflammation and EER. Pepsin detection indicates the presence of gastric juice in the middle ear, with all of its associated negative effects. The advantage of pepsin detection when compared with pH monitoring is that pepsin in tissues and fluids can be detected even in the absence of reflux within the previous several days [16]. The first report on the presence of pepsin in the middle ear effusions of children with OME was published by Tasker et al. [20]. The authors reported that 59/65 (90.8%) effusion samples from children with OME were positive for pepsin or pepsinogen (measured by ELISA). Moreover, pepsin activity was shown by enzymatic assay in 19/65 (29.2%) effusion samples. The levels of pepsin/pepsinogen protein in effusion samples were up to 1000 times higher than in serum samples. This indicates that the pepsin in the effusion samples was of gastric origin [20].

Several recent studies have shown that pepsin is present in 56–90% of middle ear specimens in children with OME [6,20], and active pepsin has been detected in 14.5–29% of specimens [5,9,20]. This percentage range is wide, and it may reflect the influence of many factors, such as the number of patients included, age of the patients, the method used for detection and concurrent diseases (e.g., a higher prevalence of reflux in children with bronchial asthma). There is evidence that gastroesophageal reflux occurs in approximately two-thirds of infants at 4 months of age but decreases as the child matures [21–23]. This is consistent with the results of He et al., who reported that the highest rate of samples

positive for pepsin activity occurred in children <1 year old (31%), compared to 14.5% positive samples for the study as a whole [5].

The ideal method for diagnosis of EER in patients with OME would be the one, which could predict response to antireflux treatment. Unfortunately such a method does not exist at present. It can only be assumed that patients with more serious EER or more pepsin present in the fluids or tissues are at higher risk for developing OME and would be potential candidates for antireflux treatment.

Another consideration is whether detection of the presence of pepsin is more appropriate than measuring its activity, or vice versa. At first sight, pepsin activity measurement seems to be more appropriate, because it indicates the actual risk of middle ear mucosa. However, it is known that pepsin is inactive but stable at pH 7.0, and that it can be reactivated upon re-acidification, retaining  $79 \pm 11\%$  of its original activity at pH 3.0 [24]. Therefore, detection of the presence of pepsin would seem to be more suitable for diagnostic purposes, as pepsin activity can vary over time according to the actual pH.

According to the latest reports, Peptest seems to be a convenient, office-based, non-invasive, quick, and inexpensive technique for EER diagnosis. It is possible to get results within 30 min, and it is not necessary to send samples to a laboratory for analysis. The detection limit of Peptest is  $0.016 \mu\text{g/ml}$  pepsin. Positive and negative predictive values for detection of pepsin in saliva using Peptest for reflux disease (esophagitis or abnormal pH testing) are 87% and 78%, respectively [25]. The sensitivity and specificity of the assay was 87%, as ascertained by in vitro bench testing.

This is the first study, which used Peptest for detection of pepsin in middle ear effusions. The presence of pepsin was detected in 32.2% middle ear specimens. The activity of the pepsin was not measured, as it can vary over short timespans, as mentioned above. Pepsin was present in fewer middle ear effusions when compared with previous studies, which detected pepsin in 56–90% of middle ear specimens. This can be explained by the fact that ELISA and Western blotting can detect even the smallest quantities of pepsin. Therefore the sensitivity of Peptest is lower and a higher rate of false negative specimens could be expected. However, in clinical practice, it is desirable to select patients with the most severe reflux who would potentially best respond to antireflux therapy. The percentage of patients in our study identified as having pepsin in their middle ear effusion using Peptest was 31.8%. This seems to be a reasonable proportion of patients who could hypothetically be put on antireflux therapy.

Two limitations of this study should be mentioned. First, the study would be “stronger” if another diagnostic technique for pepsin detection (ELISA, Western blot) or detection of EER were to be performed simultaneously (e.g. pH monitoring). Second, the response to antireflux therapy should be examined in the future to prove the usefulness or otherwise of Peptest in the selection of patients suitable for antireflux therapy. This should be the subject of ongoing studies.

## 5. Conclusions

Peptest can be used for diagnosis of the presence of pepsin in the middle ear fluid. Pepsin was detected using Peptest in 1/3 of middle ear specimens obtained from children with OME. These patients probably have more severe reflux and would potentially be candidates for antireflux therapy. However, this has to be confirmed in further studies.

## Conflict of interest statement

The authors declare that there is no actual or potential conflict of interest in relation to this article. No benefits in any form have

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