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Helicobacter pylori detection and clinical symptomatology of gastroesophageal reflux disease in pediatric patients with otitis media with effusion

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ABSTRACT

Objectives: The study aimed to demonstrate *Helicobacter pylori* presence in otitis media with effusion (OME) and its association with symptomatology of gastroesophageal reflux disease (GERD).
Methods: In a cohort study, 69 effusions were collected during tympanostomy tube insertion for *H. pylori* detection using PCR and ELISA. Validated questionnaires were performed according to age for clinical diagnosis of GERD; chi-square ×2 statistical analysis was made.
Results: Eight of the 69 ear effusions (5.7%) were positive for *H. pylori* detection using ELISA. Two patients (2.9%) had positive results for *H. pylori* detection using ELISA and PCR. These eight patients had positive results too in GERD questionnaires. None of the patients with negative/suspect questionnaires had positive results for *H. pylori*. We found statistical association between the results of ELISA, PCR and questionnaires (×2, $p = 0.001$).
Conclusions: The *H. pylori* presence in effusions varies widely, in our population the frequency was lower than other reports. We found strong association between *H. pylori* in effusions and positive GERD questionnaires. The bacterium role in OME chronicity is not clear, but this study supports the GERD participation in OME pathogenesis.

1. Introduction

Otitis media with effusion (OME) is defined as the presence of fluid in the middle ear without signs or symptoms of acute infection, being a common pathology of patients of pediatric age and a major cause of conductive hearing loss in this group [1,2]. It has been estimated that by the age of 3, almost every child has experienced at least one OME episode [2–4]. An important fact is the lack of successful medical management for OME, being the surgical drainage the option of treatment when precise indications exist [4,5]. A multifactorial etiology has been advocated with gastroesophageal reflux a possible associated factor [4,6–9]. It seems likely that gastric reflux to the nasopharynx and from here to the middle ear is possible due to eustachian tube angulation and functional immaturity in pediatric patients. The resulting inflammation creates the ideal conditions for obstruction and bacterial biofilm accumulation [3,7–10]. In 2002 Tasker et al., measured pepsin concentrations in middle ear effusions from 54 children using ELISA and enzyme activity assays, finding in 45 (83%) pepsin/

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pepsinogen concentrations up to 1000-fold greater than those in serum, giving support to the relationship between reflux of gastric juice in middle ear effusions and OME [7]. Another indirect measure of this relationship has been the finding of *Helicobacter pylori* DNA by PCR, in middle ear effusions of between 16.3% and 35% according to different studies [11–16]. The presence of these bacteria has been demonstrated by other methods such as immunohistochemistry and CLO test [17,18]. A systematic review of the relationship between *H. pylori* and otitis media with effusion (203 patients) and more recent revisions concluded that the precise role of the bacteria in the pathogenesis of middle ear effusions and the relationship with gastroesophageal reflux is not clear [19,20]. Our interest was to study the association of symptomatology of gastroesophageal reflux disease (GERD) and the presence of *H. pylori* in middle ear effusions.

2. Methods

We design a prospective cohort, including all pediatric patients of any age, with OME who require a tympanostomy tube. The patients treated in the Department of Pediatric Otorhinolaryngology, High Specialty Medical Care Unit of the Pediatric Hospital of the *Centro Médico Nacional Siglo XXI*, a tertiary-care hospital of the *Instituto Mexicano del Seguro Social* (IMSS) in Mexico City, between March 2011 and July 2014. IMSS is the largest health and social security institution in Latin America and the most important in Mexico where 35 million people receive attention [21]. The study was approved by the hospital ethics board (R-2011-3603-1). Written informed consent was obtained from the participants' parents.

OME diagnosis was made based on clinical examination (otomicroscopy), audiometric, and tympanometric testing according to the clinical guidelines [2]. All the patients included in the study were candidates for tympanostomy tubes according to the recommendations of tympanostomy tubes in children clinical practice guideline (Table 1) [5]. In the preoperative evaluation, two instruments for GERD diagnosis were applied to all patients according to age. The modified infant's questionnaire for GERD by Orenstein (I-GERQ-R) for children under 7 years and for those over 7 years, the Nelson questionnaire. Both instruments were validated for clinical diagnosis of GERD with approximately 74% of sensitivity and specificity of 81% according to different reports and these instruments are considered useful as screening clinical test in children to who GERD is suspect [22–24].

In the case of I-GERQ-R, this is a useful instrument with robust evaluative properties for tracking symptoms during clinical trials [22]. The positive and negative predictive values for the I-GERQ-R score were 1.00 and 0.94–0.98 [21]. Nelson's questionnaire was validated in a survey by the Pediatric Practice Research Group (a research consortium of practices in Chicago and surrounding area affiliated to Children's Memorial Medical Center and includes urban, suburban and semirural offices). Reliability of the survey instrument was probe by test-retest consistency using proportion of agreement with a median proportion for agreement of 0.93 [24].

We excluded from the study those patients with previous antibiotic therapy (3 months before) and those of whom we did not obtain sufficient sample for realization of PCR and ELISA (minimum of 0.2 ml).

2.1. Technical procedures for PCR and ELISA

All the samples were obtained from middle ear with sterile technique and preserved under 4 °C. Immunoglobulin G (IgG) antibodies against *H. pylori* whole-cell antigens were tested in samples using an enzyme linked immunoabsorbent assay, which was previously validated for the Mexican population [25]. A pool of whole-cell antigen preparations was obtained from three Mexican strains of *H. pylori* and attached to the plates. Samples were tested in a 1:1000 dilution. Next a 1:1000 dilution of antihuman IgG monoclonal antibodies conjugated to alkaline phosphatase (Southern Biotech, Birmingham, AL, USA) was applied. A 1 mg/ml solution of p-nitrophenylphosphate was used as substrate, and absorbance was read at 405 nm. All samples were analyzed by duplicate; the final value was given by the average of the two measurements. Patients were considered as positive for *H. pylori* infection when ELISA units were >1.0. Total genomic DNA was obtained from samples, using the commercial Wizard method (Promega Corporation, Madison, WI) according to the manufacturer's instructions. DNAs from *H. pylori* strains 26695 (ATCC 700392) and Tx30a (ATCC 51932) were prepared for use as controls. All PCR mixtures consisted of 100 ng of chromosomal DNA template, 1× PCR buffer, 1.5 *mM MgCl2*, a 0.2 mM concentration of each deoxynucleoside triphosphate (Boehringer Mannheim, Germany), 25 pmol of each primer, and 1.25 units of *Taq* DNA polymerase (Invitrogen, Life Technologies, Brazil) in a final volume of 25 μl. PCRs were performed in a thermal cycler (GeneAmp PCR system 9700; PE Applied Biosystems). *H. pylori* DNA was detected with RNA 16SF5′GCTAAGAGATCAGCCTATGTCC3'and RNA16SR5′TGGCAATC AGCGTC.

AGGTAATG-3'oligonucleotides that amplify a segment of the rRNA 16S gene. Each reaction mixture was amplified for 39 cycles as follows: 1 min at 94 °C, 1 min of annealing at 55 °C), and 2 min at 72 °C [26]. The size of the product was 522 bp. The PCR-amplified products were analyzed by electrophoresis on 1.5% agarose gels. The gels were stained with ethidium bromide and examined under ultraviolet light.

3. Results

We included a total of 50 patients, 31 cases with unilateral and 19 cases with bilateral disease (38 ears) for a total of 69 ears studied. There were 35 male patients (70%). The median age was 5 years (1–13 years). Only 10 patients (20%) had previous

Table 1
Resumed action statements for tympanostomy tube insertion indications according to tympanostomy tubes in children clinical practice guideline [5].

Statement	Action
Chronic bilateral OME with hearing difficulty	Clinicians should offer bilateral tympanostomy tube insertion to children with bilateral OME for 3 months or longer and documented hearing difficulties.
Chronic OME with symptoms	Clinicians may perform tympanostomy tube insertion to children with bilateral OME for 3 months or longer AND symptoms that are likely attributable to OME that include, but are not limited to, vestibular problems, poor school performance, behavioral problems, ear discomfort, or reduced quality of life.
Recurrent acute otitis media with middle ear effusion	Clinicians should offer bilateral tympanostomy tube insertion to children with recurrent acute otitis media who have unilateral or bilateral middle ear effusion at the time of assessment for tube candidacy
Tympanostomy tubes in at risk children	Clinicians may perform tympanostomy tube insertion in at risk children with unilateral or bilateral OME that is unlikely to resolve quickly as reflected by a type B (flat) tympanogram or persistence of effusion for 3 months or longer.