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Acute Otitis Media Severity: Association with Cytokine Gene polymorphisms and other Risk Factors

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Abstract

Background—We have previously shown an association between polymorphisms of proinflammatory cytokine genes and susceptibility to upper respiratory tract infection and acute otitis media. It has not been known whether polymorphisms or risk factors are associated with the severity of acute otitis media.

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Objective—To evaluate the influences of proinflammatory cytokine gene polymorphisms and other risk factors on severity of acute otitis media following upper respiratory infection.

Methods—In a prospective, longitudinal study, children aged 6-35 months were followed for one year for occurrences of upper respiratory tract infection and acute otitis media. Children were studied for TNFα^{-308}, interleukin (IL)- 6^{-174} and IL-1 ß$^{+3953}$ polymorphisms, taking into account age, gender, race, family history of otitis, tobacco smoke exposure, breast feeding, day of upper respiratory tract infection at the time of diagnosis and pneumococcal vaccine status. Symptoms and signs of acute otitis media were graded according to a validated scale. The association between acute otitis media clinical severity, polymorphic genotypes, and risk factors was analyzed using statistical models that account for multiple episodes of acute otitis media per child.

Results—A total of 295 episodes of acute otitis media in 128 subjects were included. More severe acute otitis media symptoms were associated with young age (P=0.01), family history of acute otitis media (P=0.002), tobacco smoke exposure (P=0.008), and early diagnosis of otitis after onset of upper respiratory tract infection (P=0.02). Among children with a bulging or perforated tympanic membrane (206 episodes, 104 subjects), those who had the IL- 1 ß$^{+3953}$ polymorphism, experienced higher symptom scores (P<0.02).

Conclusion—This is the first report of the association between risk factors and acute otitis media severity. Risk factors such as tobacco smoke exposure and a positive family history appear to be more significantly associated with acute otitis media severity than proinflammatory gene polymorphisms. Clinical severity may be an important factor contributing to the incidence and costs of acute otitis media, because children with more severe symptoms might be more likely to be brought for a medical visit, receive a diagnosis of acute otitis media, and be prescribed an antibiotic.

Keywords

acute otitis media; interleukin; cytokine; polymorphism; tobacco smoke; tympanic membrane

Introduction

Acute otitis media (AOM) is one of the most common infections in children. It is the leading cause of illness-related clinic visits, the consumption of antibiotics, and surgery [1,2]. As many as 83% of all children experience one or more episodes by the age of three years [3]. The etiology of AOM is multifactorial, involving the interaction of the host with infectious agents and environmental factors. Episodes of AOM can be reduced by vaccines, breast feeding, avoiding large group day care, and preventing exposure to tobacco smoke. Widespread use of pneumococcal vaccine has reduced AOM episodes and lessened the need for surgery [4].

Genetic factors have been associated with increased susceptibility to AOM in children, as has been demonstrated in studies of twins, triplets and related family members [5-9]. Genes that control cytokine production can be involved in susceptibility and severity of airway inflammation [10]. Our studies have shown IL-1ß, IL-6 and TNFα in the nasopoharyngeal secretions during URI, and increased levels of IL-1ß correlate with transition from URI to AOM [11]. In addition, we have shown a relation between risk for AOM episodes complicating URI and TNFα^{-308} polymorphisms [12]. TNFα^{-308} and IL-6^{-174} polymorphisms are associated with recurrent AOM [13]. Despite these advances, it has not been known if genetic and environmental risk factors can be associated with severity of AOM. Such information is needed, as severity of AOM is considered an important variable in the current AOM treatment guidelines [14]. In the present study, we have evaluated the

clinical severity of AOM in relation to cytokine gene polymorphisms and environmental risk factors.

Methods

Clinical evaluation

The aim of the study was to describe the incidence and characteristics of AOM complicating URI in order to understand risk factors associated with the development of AOM [15]. The study was performed at the University of Texas Medical Branch at Galveston and was approved by the UTMB Institutional Review Board. Written informed consent was obtained from the parent or guardian. The study was performed from January 2003 through March 2007. Healthy children aged 6-35 months were recruited from the primary care pediatrics clinics at the University of Texas Medical Branch. Children with anatomic and physiologic defect of the nasopharynx or ear (including tympanostomy tubes), or with major medical conditions were excluded.

Demographic and risk factor data were obtained by parent questionnaire. Risk factor variables included (a) child care: home, home day care, or day care center, (b) family history of AOM: any first-degree family member (mother, father, siblings) with history of AOM, (c) breastfeeding: < one week versus ≥ one week (62 subjects breast fed > 2 weeks), and (d) tobacco smoke exposure in the child's principal residence: none versus any. Heptavalent protein-conjugate pneumococcal vaccine status was obtained from the medical record.

Each subject was followed for one year to study occurrences of URI and AOM. Subjects were seen by a study physician as soon as possible after the onset of URI symptoms (nasal congestion, rhinorrhea, cough, and/or sore throat, with or without fever) and followed up 3-7 days later. Study personnel provided 2 home visits and performed tympanometry during weeks 2-3 of URI. In addition to these URI-associated visits, parents were also advised to bring the child for examination whenever they independently suspected the child to have symptoms of AOM. Children diagnosed with AOM were observed or given antibiotic therapy consistent with standard of care.

Definition of AOM, severity of TM inflammation

AOM was defined by acute onset of symptoms, signs of tympanic membrane inflammation, and middle ear effusion. All children had URI symptoms. Other recorded symptoms included presence or absence of: earache, fever, poor feeding, restless sleep, and irritability. Signs of tympanic membrane inflammation included erythema, opacification, and bulging [16]. The presence of middle ear effusion was documented by pneumatic otoscopy and/or tympanometry or the observation of an acutely draining ear due to perforation. Tympanic membrane appearance was categorized as non severe (erythema, with or without opacification, not bulging) or severe (erythema, full/bulging or acute perforation).

AOM symptoms

Symptoms were quantified using a previously described five-item parent questionnaire (ETG-5) that assessed earache, fever, poor feeding, restless sleep, and irritability [16,17, 18]. Each item was evaluated on a scale ranging from zero (no symptoms) to three (severe symptoms). ETG-5 total score was the sum of the items (range 0-15). In children diagnosed with AOM, acute otitis media faces scale (AOM-FS, 18) was also used for the parent to describe the child's symptoms in the previous 24 hours.

Cytokine gene polymorphism analysis

Deoxyribonucleic acid (DNA) was extracted from peripheral blood white cells or buccal epithelial cells of enrolled children, as previously described [13]. Polymerase chain reaction (PCR) was performed on the extracted DNA with the use of respective cytokine primer sets that spanned the single nucleotide polymorphism (SNP) sites (TNFα^{-308}, normal G/G, heterozygous G/A, polymorphic homozygous A/A; interleukin IL- 6^{-174} , normal G/G, heterozygous G/C, homozygous C/C; IL-1 ß$^{+3953}$, normal C/C, heterozygous C/T, homozygous T/T). The resultant PCR products were digested with polymorphic site-specific enzymes. All SNP's that were identified by PCR and restriction fragment-length polymorphism were confirmed by sequencing (DNA sequencer, Applied Biosystems, 3130, Carlsbad, CA). The study of polymorphisms yielded 3 genotypes for each cytokine: homozygous “normal” (low cytokine producing) and homozygous and heterozygous polymorphic (high cytokine-producing). For data analysis, children were considered to be polymorphic if they were either homozygous or heterozygous for the polymorphic genotypes.

Statistical analysis

The primary outcome variable was the total symptom score (ETG-5), which had a possible range of 0-15, and was approximately normally distributed in our models. Using other distributions (e.g. negative binomial) did not change the results or conclusions. Each child could have several URI episodes and hence subjects contributed varying amounts of correlated data to the study. The statistical approaches to data analysis accounted for multiple episodes of URI, which resulted in clusters of correlated data from each subject. We used a class of models called repeated measures general linear mixed models (GLMM) for parameter estimation and P values derived from the GENMOD procedure in SAS® (Cary, NC) specifying a normal distribution working correlation structure. We modeled the covariance structure so that scores closer together in time are considered more correlated than symptom scores further apart in time (AR-1 covariance structure). The model provides a parameter estimate (see Table 2) which, for a given numeric variable such as “days” or “age in months”, is the change in ETG-5 symptoms score attributable to a one unit change in that variable. If the variable is categorical, such as environmental smoke exposure “yes” or “no”, then the parameter estimate is the simply the difference in ETG-5 score between the two groups, “smoke exposed” and “not smoke exposed”. Associations between polymorphic gene status and risk factors were first analyzed for all episodes. A secondary and exploratory analysis was then conducted only for episodes with moderate/severe inflammation of the TM (diagnosis of AOM with full or bulging TM). The 16 episodes in which a perforated TM was noted were included in the moderate/severe group.

Results

Clinical and demographic data

Between January 2003 and March 2007, 294 subjects had 1295 episodes of URI and 440 episodes of AOM. Included in this analysis were data from 128 children with 295 episodes of AOM that were evaluated by the study team prior to antibiotic treatment. Excluded from the analysis were AOM episodes diagnosed by non-investigator clinicians, episodes when AOM was confirmed by the study team after antibiotic was initiated, or episodes with missing clinical data or unavailable polymorphism results. Table 1 describes the subjects’ demographic, genetic, and risk factor variables, including pneumococcal vaccine status. Gender distribution showed a slight predominance of males. A minority of children attended any day care. Family history of otitis media was common. About half of the subjects had been breast fed. Mean age was 17.2 months at the time of enrollment.

AOM diagnosis, severity scores

AOM was diagnosed by the investigators most frequently between days 5 and 6 of the URI. All subjects had URI symptoms. Investigators described the TM inflammation as severe (full/bulging) in 70 percent (206/295) of AOM episodes and non severe (not bulging) in 30 percent (89/295). Mean ETG-5 score for the 295 AOM episodes was 3.4 ± 2.7 (median=3, range 0 - 11). In 43 episodes, ETG-5 symptom score was zero; in 26 of these the parents reported the presence of symptoms by AOM-FS; in 11 of these episodes AOM-FS data were not available.

Table 2 summarizes results of the analysis of symptom scores relative to age, gender, environmental risk factors and polymorphism. These results show statistically significant associations between AOM symptom severity and young age, a family member with a history of chronic/recurrent AOM, household tobacco smoke exposure, and early diagnosis following the onset of URI.

In a further analysis, looking for any correlation between signs of TM inflammation, clinical symptoms and risk factors, we included only episodes in which the worse ear was observed to have severe inflammation (full/bulging TM or acutely perforated TM with drainage, n=206 episodes in 104 subjects). In this analysis, IL-1 $ß^{+3953}$ polymorphism was associated with a higher symptom score (P = 0.02). Compared to a child 12 months older with no risk factors, a child with all risk factors, would be estimated to experience an ETG-5 score 1.0 point higher, which is equal to 0.38 of a standard deviation.

We also evaluated the relation between tympanic membrane inflammation scores (OS-8) and risk factor variables in the model. Results did not show associations: age (P=0.57), gender (P=0.41), Race (P=0.49 to 0.76), family history of AOM (P=0.59), breast fed (P=0.14), tobacco smoke exposure (P=0.58), day of URI at time of diagnosis (P=0.95), heptavalent pneumococcal vaccine status (P=0.97), $TNFα^{-308}$ polymorphic (P=0.57), IL-1 $ß^{+3953}$ polymorphic (P=0.27), and IL- 6^{-174} polymorphic (P=0.12). These data were also analyzed using just the episodes with severe TM inflammation (n=206 episodes, n=104 subjects), and no significant associations between tympanic membrane inflammation scores and risk factor variables were noted.

Discussion

There have been recent reports on standardized assessment of AOM severity [18-21]. In addition to the number and timing of AOM episodes, AOM symptom severity can be an important factor when considering quality-of-life issues for parents and children. AOM symptom severity may predict office visits for AOM, since some parents may not seek medical attention for children with mild symptoms. Severity may also be related to antibiotic use, since children with non-severe AOM may become candidates for watchful waiting or a safety net antibiotic prescription [22-24].

AOM is a multifactorial disease, involving genetic, environmental factors, anatomical variations, pathogen, and host response. In a meta-analysis [25], the following factors increased the relative risk for an AOM event: a positive family history, day care attendance, pacifier use, tobacco smoke exposure. Breast feeding for at least three months reduced the risk of AOM. To our knowledge, the current study is the first to report associations between AOM clinical symptoms, environmental risk factors, and cytokine gene polymorphisms.

Since it has been shown that URI precedes AOM [15,26,27], preventive efforts need to detail the immunological events that affect the transition from URI to AOM. Identification of important genetic markers might support focused approaches to prevent and manage

AOM in children whose genetic biomarkers indicate they are at risk. A variety of interventions might be considered for this subgroup of high risk children, such as (a) family-specific educational interventions to change known environmental factors, (b) developing cytokine-specific anti-inflammatory agents, (c) preventing URI through the use of viral and/or bacterial vaccines, and (d) prevention of nasopharyngeal colonization by pathogenic bacteria with vaccines or interfering agents.

Exposure to environmental tobacco smoke has been repeatedly associated with respiratory illness in children, and specifically with early onset and recurrent AOM. Smoke exposure may work through several mechanisms including ciliostasis, goblet cell hyperplasia, mucus hypersecretion, and the reduction of interfering bacterial species. Smoke exposure causes inflammation of mucosal surfaces of the nasopharynx, eustachian tube and the middle ear, which may result in epithelial injury predisposing to bacterial colonization [28-29]. Greenberg et al [30] found that *S. pneumoniae* carriage rates were higher in mothers who smoked and among children exposed to smoking. Increased bacterial colonization is correlated with higher rates of AOM development during URI [31-32]. It is thought that exposure to tobacco smoke may enhance binding of pathogenic bacteria to respiratory epithelial cells. Interestingly, AOM episodes appear to be more frequent in the children of mothers who smoked during pregnancy, even when controlling for post-natal smoke exposure [33, 34].

Our study provides further evidence for the importance of heredity in the development and clinical presentation of AOM. Prospective studies have shown that siblings of children with chronic/recurrent otitis media have an increased risk of otitis [3, 5-7, 35, 36]. Progress has been made in identifying locations on specific chromosomes that may be involved in susceptibility to chronic or recurrent otitis media [8,9].

Polymorphisms of cytokine genes, proteins that regulate a large number of biological events, have been shown to influence susceptibility to OM in human and animal studies. It is known that cytokines such as IL-1ß, IL-6, and TNFα are actively induced in nasal secretions of children during viral URI, levels of which, in the nasal secretions, may be associated with the degree of inflammation and/or recovery from infection. We have previously reported [13] that TNFα^{-308} and IL-6^{-174} genotypes were associated with increased risk for recurrent otitis media and tympanostomy tube surgery. Similarly, Emonts el al [37] reported an association between polymorphisms in immunoresponsive genes TNFα, IL-6, IL-10, and TLR-4. In the same population as in the present study, we have shown an association between higher IL-1β concentrations in nasopharyngeal secretions of children with URI and AOM development after URI [11]. Our present data on the association between symptom severity and high-cytokine-producing IL-1ß$^{+3953}$ polymorphic genotype in a subset of children with more severe tympanic membrane involvement further support the significant role of IL-1ß in the inflammatory process during the transition from URI to AOM.

Limitations of our study were the relatively small numbers of polymorphic children enrolled and the wide clinical spectrum of AOM, including mild and early cases in our population. The design of this study was to capture AOM complicating URI: we routinely examined the child for AOM twice in the first week of URI and followed them closely thereafter. We, therefore, were likely to have seen more cases of early and mild AOM that may not have been brought to medical attention had the child not been on a research study.

In conclusion, we have shown associations between AOM symptom severity and important risk factors: these include young age, early diagnosis of AOM during an episode of URI, family history of AOM, exposure to environmental tobacco smoke, and IL-1 ß$^{+3953}$ polymorphism. These results parallel previous studies that have shown associations between

environmental risk factors, genotype, and AOM episodes. The results provide a basis on which to identify and target high risk children for new preventive and/or treatment regimens.

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Abbreviations

URI	Upper respiratory infection
AOM	Acute otitis media
TM	Tympanic membrane
TNF	Tumor necrosis factor
IL	interleukin
ETG-5	Ear Treatment Group 5 symptom questionnaire
DNA	Deoxyribonucleic acid
PCR	Polymerase chain reaction
SNP	Single nucleotide polymorphism
G	Guanine
A	Adenine
T	Thymine
C	Cytosine
CI	Confidence Interval
P	Probability

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