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Do *Staphylococcus aureus* superantigens play a role in the pathogenesis of otitis media with effusion in children?

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ABSTRACT

Objectives: Staphylococcal enterotoxins (SEs), acting as superantigens, have been reported to be involved in the pathogenesis of chronic inflammatory diseases of the upper and lower airway. There has been no previous study investigating the role of SEs in otitis media with effusion (OME). Therefore, this study was designed to analyze middle ear aspirates from children with and without OME for the presence of SEs.
Methods: Middle ear aspirates were obtained from 24 patients and 24 controls. All samples were processed for bacterial culture and detection of five staphylococcal SEs (SEA, SEB, SEC and SED) and toxic shock syndrome toxin-1 using the Rapid Latex Agglutination Test.
Results: In bacterial culture assays, six samples (25%) of the study group and five samples (20.8%) of the control group showed bacterial growth. At least one SE was demonstrated in 6 of 24 patients and in 3 of 24 controls. There was no statistically significant difference between the two groups with respect to the presence of SEs.
Conclusion: Although there is evidence that SEs have a potential role in the pathogenesis of chronic inflammatory diseases, there is no evidence that the inflammation process is initiated by SEs in patients with OME.

1. Introduction

Infection agents such as bacteria, and their breakdown products, may give rise to inflammatory stimuli in patients with OME [1]. *Streptococcus pneumoniae*, *Haemophilus influenza*, and *Moraxella catarrhalis* are the most commonly cultured bacteria from up to 25% of middle ear effusions (MEE) [2]. However, 40–60% of cultured MEE in general bacterial culture tests are negative, likely because of the presence of slow-growing bacteria, intracellular organisms such as mycoplasma, or organisms such as viruses and anaerobic bacteria [3]. Moreover, polymerase chain reaction (PCR), which has a superior ability in detecting bacterial species, has demonstrated bacterial DNA in over 94% of MEE [4].

In many studies, specifically *Staphylococcus aureus* had the highest isolation rate in patients with OME, even after excluding the possibility of OME contamination [5–7]. *S. aureus* also forms biofilms and has previously been found in middle ear diseases [7,8]. Although the pathogenicity of *S. aureus* is firmly connected with the production of coagulase enzymes, these organisms also produce two types of toxins with superantigen (SAg) properties: *S. aureus* enterotoxin (SE), which has more than 24 antigenic types (SEA, SEB, SEC,. . .SEX) and toxic shock syndrome toxin-1 (TSST-1) [9]. There is increasing evidence that microbial SAgs, especially SEs, are important in raising inflammation in atopic and nonatopic patients. Kanazawa et al. reported that SE is an important factor in causing eosinophilic inflammation in patients with eosinophilic otitis media (EOM), which is characterized by an increased level of antigen-specific IgE antibody against SEs in MEE samples [10]. Most studies have demonstrated that SEs have a potentially important role in the pathogenesis of chronic inflammatory diseases such as allergic rhinitis, nasal polyposis, atopic dermatitis and asthma [11–14]. To the best our knowledge, an analysis of SE in the OME of patients has not previously been reported in the English language literature. Therefore, the aim of the present study was to investigate whether SE may play a significant role in the pathogenesis and etiology of OME.

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2. Materials and methods

2.1. Patients

This prospective, controlled study including 48 children (18 girls and 30 boys) was conducted at the Department of Otolaryngology of Sakarya University Medical Faculty between February 2014 and July 2015. The study group consisted of 24 children with OME who were planned to have ventilation tube insertion. The diagnostic criteria for OME were as follows: (1) Tympanometry showing type B or C tympanograms, (2) Otomicroscopy showing the presence of an amber-colored tympanic membrane or the presence of an air-fluid level behind the transparent membrane for at least three months without response to antibiotic treatment. In the control group, patients with recurrent tonsillitis or chronic tonsillitis were planned to have tonsillectomy plus myringotomy. The indications for myringotomy were a suspected hearing loss, a "popping" in the ears, a "plugged" feeling or a poor correlation between otomicroscopic findings and a type C tympanogram of the suspected ear. Following myringotomy, 24 children were enrolled in the control group upon the determination of an absence of MEE intra-operatively. Neither complications nor ear infections occurred in the study or control groups at three months follow-up. The exclusion criteria were the presence of a previous adenotonsillectomy, middle ear operation and/or ventilation tube insertion. The families of both groups were asked to respond to a questionnaire on passive smoking, allergies and atopy, socioeconomic status, methods of childbirth, taking care of pets, and family history of otitis media. This study was approved by the local Ethical Committee. Written informed consent was obtained from each patient after discussion of the surgical procedure's full details and the underlying aim.

2.2. Sample collection

Under general anesthesia, a standard anterior-inferior myringotomy was performed for placement of the ventilation tube and/or collection of the middle ear aspirate after the ear canal had been cleaned using povidone iodine solution. In the control group of patients who were without effusion, sterile saline (1 mL) was injected into the middle ear with the help of a 27 gauge needle to obtain control samples. Both the MEE and the control samples were collected aseptically with a JuhnTym–Tapaspirator/collector (XomedTreace Products, S.K. Juhn, U.S.A.) and sent to the Department of Infectious Disease.

2.3. Bacterial culture, the search for enterotoxins

Samples were stored at −70 °C in a deep freezer until the study day. All samples were processed for bacterial culture and detection of five staphylococcal SE (SEA, SEB, SEC, SED and TSST-1) using the Rapid Latex Agglutination Test with commercially available kits (SET-RPLA-Test, TST-RPLA-Toxin detection test; Oxoid, Tokyo, Japan). On the study day, they were inoculated with both sheep 5% blood agar and EMB agar in accordance with routine procedures. Microorganisms were identified according to biochemical properties with conventional methods. Toxin detection tests were performed according to the manufacturer's recommendations. Briefly, a mixture was made in microplates (V-well) with a 25 M pipette according to the manufacturer's recommendations. Positive and negative controls were used for each test. After this procedure, plates were left undisturbed on a vibration-free surface at room temperature for 20 to 24 h. Each well was examined in each row for agglutination against a black background. The sensitivity of this test in detecting TSST-1 and SE was approximately 2 ng/ml.

2.4. Statistical analyses

To calculate the necessary sample size, effect size was calculated as 0.8 since there was no information from previous studies. The sample size was calculated as 19, assuming an effect size of 0.8, type I error (alpha) = 0.05, and type II error (1-power) = 0.33 for statistical significance. Power analysis was performed using G*Power (G*Power version 3.1.9, Franz Faul, Universitat, Kiel, Germany). However, a sample size of 24 in each group was obtained. Shapiro–Wilk test was used for the normality test. Descriptive statistics for age variable are shown as mean $\pm$ standard deviation (minimum, maximum). Frequency (n) and percentage (%) are given for categorical variables. To investigate the difference between the study and control groups with respect to numeric variables, Independent samples t test was performed after determining whether the variables met the necessary assumptions.

Pearson Chi-Squared test and Fisher exact test (when any expected counts were less than 5 for 2×2 contingency tables) were used for categorical variables. If there were not enough patients for any cell, only the frequency was used. Data were analyzed with IBM SPSS Statistics 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp. and MS-Excel 2007). Type I error was taken as $\alpha = 0.05$ for statistical significance.

3. Results

There were 14 (58.3%) boys and 10 (41.7%) girls in the control group while there were 16 (66.7%) boys and 8 (33.3%) girls in the study group. Gender distribution was similar between the two groups ($\chi^2 = 0.356$; $p = 0.551$). The mean age was similar between study and control groups ($p = 0.999$). In the study and control groups, the mean age was 7.1 $\pm$ 2.4 (4; 13) years and 7.1 $\pm$ 2.1 (2; 11) years, respectively.

As shown in Table 1, no differences were detected between the two groups for frequency distribution of socioeconomic situation, passive smoking, atopy, family allergy atopy, taking care of pets, methods of childbirth and family history of otitis media. The proportion of family allergy atopy's present was 41.7% ($n = 10$) in the study group and 37.5% ($n = 9$) in the control group ($\chi^2 = 0.087$; $p = 0.768$). There was no statistically significant difference between the groups with respect to family history of otitis media ($\chi^2 = 1.333$; $p = 0.248$). The frequency of otitis media in the family was 14 (58.3%) in the study group while it was 10 (41.7%) in the control group.

Table 2 summarizes the frequency distribution of SEA, SEB, SEC, SED and TSST-1 between the two groups, which was not significantly different ($p > 0.05$). There were no positive results of SEA, SEB or SED in the control group. According to standard culture assay, growth of bacteria was detected in six samples (25%) of the study group and five samples (20.8%) in the control group. Three positive growths were identified as *S. epidermidis*, one was *S. aureus*, and two were others in the study group. Two positive growths were *S. aureus*, one was *S. epidermidis*, and two were others in the control group. There were no statistically significant differences between the groups with respect to the presence of reproduction ($\chi^2 = 0.118$; $p = 0.731$). In both groups, the culture results were the same (Table 3).

4. Discussion

Different factors have been proposed in the etiology of OME, all of which are probably related to an increased tendency to infection [1]. In some studies, the bacterium implicated in OME was *S. aureus*, rather than just the typical bacteria studied in the past (*S. pneumoniae, H. influenza, and M. catarrhalis*) [7–9]. Interestingly,