ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Study Of Sleep Disordered Breathing And Otitis Media With Effusion In Pediatric Cases.

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ABSTRACT

Streptococcus pneumonia, one of the main bacteria invading nasopharyngeal adenoids, often causes AOM in youngsters. Recent studies suggest a connection between the elimination of pneumococcal colonization in nasopharyngeal adenoids and the immunological response mediated by IL-17A. In this work, we examined the association between the expression of IL-17A and associated genes in hypertrophic adenoid tissues from children with sleep disordered breathing (SDB) and otitis media with effusion (OME), as well as their relationship to pneumococcal carriage. We actively enrolled children with SDB and OME at the Otolaryngology Department. Patients who met the requirements were enrolled. We discovered that adenoidal IL-17A and IL-17A:IL-10 mRNA levels were significantly higher in SDB patients who tested positive for pneumococcal carriage than in those who tested negative. However, the OME group did not think that these variances were noteworthy. These results suggested that a modest response to IL-17A-mediated mucosal clearance may be the cause of the prolonged or chronic pneumococcal carriage in OME patients. The results suggested that the prolonged or chronic pneumococcal carriage in OME patients might be caused by a weak response to IL-17A-mediated mucosal clearance.

Keywords: Sleep disorder, breathing disorder, OME patients, chronic pneumococcal carriage

https://doi.org/10.33887/rjpbcs/2022.13.5.35

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ISSN: 0975-8585

INTRODUCTION

AOM in children is typically brought on by Streptococcus pneumonia, one of the principal pathogens colonizing nasopharyngeal adenoids. Recent research points to a correlation between the immune response mediated by IL-17A and the removal of pneumococcal colonization in nasopharyngeal adenoids. In this study, we looked at the expression of IL-17A and related genes in hypertrophic adenoid tissues from kids with sleep disordered breathing (SDB) and otitis media with effusion (OME) and their relationship to pneumococcal carriage. A total of 66 young individuals with adenoid hypertrophy were included. Nasopharyngeal swab and adenoid tissues were utilized to assess pneumococcal carriage and IL-17A expression during adenoidectomy. Our findings showed that the SDB patients who tested positive for nasopharyngeal pneumococcal carriage had considerably greater levels of IL-17A and IL-17A:IL-10 mRNA than those who tested negative. The OME group, however, did not find these variations to be noteworthy. These findings suggested that prolonged or chronic pneumococcal carriage in OME patients may arise from insufficient IL-17A-mediated mucosal clearance, which could further contribute to the development of AOM and OME.

METHODOLOGY

From April 2021 to May 2021, we proactively enrolled kids with SDB and OME at the Department of Otolaryngology. We enrolled patients who met the criteria below: Ages 3 to 12 years, substantial OSAS or persistent OME symptoms for longer than three months, and adenoid hypertrophy planned for adenoidectomy (tympanostomy with ventilation tube insertion) are the prerequisites. Patients who had any of the following characteristics were not included in the study: (1) use of antibiotics during the past four weeks; (2) congenital malformations; or (3) serious or persistent illnesses such cancer, diabetes, immunodeficiency, nephrotic syndrome, or autoimmune diseases.

By using a skull lateral-view radiograph taken before to surgery, the size of the nasopharyngeal adenoids was determined. The Adenoid/Nasopharynx (A/N) ratio, which was described by Fujioka et al.22, is the ratio of the length between the sphenobasioccipital synchondrosis and the posterior end of the hard palate to the length between the outermost point of the anterior convexity of the adenoid tissue (Fig. 1a). On the day before surgery and six months following surgery, the OSA-18 questionnaire23 assessed the OSAS symptom score.

The Institutional Review Board of Hospital approved this study and all participants and/or their legal guardians gave their consent before they were admitted. All study was conducted in conformity with the pertinent institutional review board rules and regulations.

Before adenoid tissue was removed during transoral endoscopic surgery, sterile swabs were used to collect bacterial cultures from the surface of the nasopharyngeal adenoid (Fig. 1b). Swabs were brought to the lab and then put on blood agar plates, Columbia colistin-nalidixic acid agar biplates, and eosin methylene blue plates using Amies Transport Medium (Copan Italia, Brescia, Italy) as the transport medium.

After that, the plates were cultured for 48 hours at 37 °C. Both traditional methods and colony morphology were used to identify the bacterial composition24. Using a different nasopharyngeal swab, multiplex polymerase chain reaction (PCR) was used to determine the serotype of S. pneumoniae as previously described25. In a nutshell, we used a QIAamp genomic DNA kit (Qiagen, Valencia, CA, USA) to extract nucleic acids from nasopharyngeal swabs, and we stored them frozen at 70 °C for later use. As previously mentioned25, 45 primer pairs for particular serotypes were created. Use of a primer pair that targets cpsA, which is present in all 90 known pneumococcal serotypes, as a positive control. The PCR conditions were as follows: 94 °C for 4 minutes of initial incubation, then 30 cycles of 94 °C for 45 seconds, 54 °C for 45 seconds, and 65 °C for 2 minutes 30 seconds. Following gel electrophoresis on a 1.4 percent agarose gel at 120 V for 45 minutes, PCR products were run. The gel was next stained with ethidium bromide and then illuminated with ultraviolet light to see it (Figure S).

Adenoidal tissues collection and processing

After being cleaned with phosphate-buffered saline (pH 7.6) and kept at 70 °C, adenoidal samples taken during transoral endoscopic adenoidectomy were then ready for real-time PCR. According to the

manufacturer's recommendations, total RNA from adenoid tissue was extracted using a Qiagen RNeasy mini kit, quantified, and stained with ethidium bromide to check for RNA integrity21. Reverse transcription was carried out using a high-capacity cDNA reverse transcription kit from Applied Biosystems in Foster City, California, USA, and random hexamer primers. As previously mentioned21, a TaqMan assay using target gene-specific primer sequences (Table 1) was carried out using an Applied Biosystems 7500 fast real-time PCR equipment. The following were the amplification conditions: 95 °C for ten minutes, followed by 45 cycles of 95 °C for ten seconds, 60 °C for twenty seconds, and 72 °C for ten seconds, and finally a final cooling time at 40 °C. To enable the quantification of gene expression, each sample was conducted in triplicate in different tubes. The mean threshold cycle (Ct) values were normalised to GAPDH before using the 2Ct method to determine the relative mRNA levels of the target genes.

Immunohistochemistry analysis

As previously mentioned21, a TaqMan assay using target gene-specific primer sequences (Table 1) was carried out using an Applied Biosystems 7500 fast real-time PCR equipment. The following were the amplification conditions: 95 °C for ten minutes, followed by 45 cycles of 95 °C for ten seconds, 60 °C for twenty seconds, and 72 °C for ten seconds, and finally a final cooling time at 40 °C. To enable the quantification of gene expression, each sample was conducted in triplicate in different tubes. The mean threshold cycle (Ct) values were normalized to GAPDH before using the 2Ct method to determine the relative mRNA levels of the target genes.

Statistical Analysis

GraphPad Prism 5 was used to carry out the statistical analysis (GraphPad Software, San Diego, CA, USA). The mean and standard deviation of the data were displayed. The Chi-squared test was utilized to compare categorical data. When comparing between two groups, continuous variables were examined using the Mann-Whitney U test or an unpaired t test, as applicable. The link between two variables was determined using Pearson's correlation coefficient (r). Statistical significance was defined as a p-value 0.05. Based on the difference between the primary outcomes in the research groups, the power was 80.5 percent.

RESULTS

A total of 66 consecutive paediatric patients with adenoid hypertrophy were enrolled for the study, comprising 28 children with OME who underwent adenoidectomy plus tympanostomy with breathing tube insertion and 38 children with SDB who underwent adenoidectomy plus tonsillectomy. The clinical features of the individuals were presented.

Pneumolysin is a protein toxin that nearly all pneumococcal strains express. It can stimulate human Th17 cells in nasopharynx-associated lymphoid tissue, which may help to eradicate pneumococcal carriage. According to Jiang et al.15, decreased clearance of S. pneumoniae and prolonged carriage may be caused by the upregulation of Treg cells in the adenoids and downregulation of Th17 cells in the adenoids. In the nasopharyngeal mucosa, Mubarak et al.16 showed a dynamic interaction between Treg and Th17 populations. This connection changes as people age and in step with the elimination of pneumococcal carriage. This result was in line with an epidemiologic investigation that revealed that pneumococcal carriage is common in young children and declines with age.

DISCUSSION

According to Hoe et al, pneumococcal carriage in paediatric nasopharynxes with low density but not high density was associated with elevated IL-17 production. Our earlier research21 found that SDB patients who had pneumococcal carriage had an increase of IL-17A.

In the current investigation, we first assessed the expression of IL-17A and its relationship to pneumococcal carriage in the adenoid tissue of children with OME. The findings showed that SDB patients who tested positive for pneumococcal carriage had considerably greater levels of IL-17A and IL-17A:IL-10 mRNAs than those who tested negative. These variations between the OME patients who had pneumococci and those who did not, however, were not statistically significant. Additionally, the levels of IL-17A mRNA were correlated

with IL-22 and its transcription factor RORt, whereas the levels of IL-10 were correlated with TGF- and its transcription component FOXP3. These findings suggest that pneumococcal carriage in nasopharyngeal adenoids in SDB children is related to an immunological response mediated by IL-17A. However, due to the negligible response of IL-17A-mediated mucosal clearance in children with OME, persistent or chronic pneumococcal carriage may happen.

Our findings in SDB patients support Hoe et alfinding .'s that low pneumococcal-carriage density is associated with higher IL-17 release. On the other hand, decreased IL-17 secretion in the OME group in the current study17 was comparable to high pneumococcal carriage density. Our results also support Gray et alwork, .'s which discovered that before pneumolysin stimulation, children with positive pneumococcal carriage had a higher frequency of Th17 cells in their tonsillar mononuclear cells than children with negative pneumococcal carriage. However, pneumococcal carriage negative children had a greater rise in the number of Th17 cells after pneumolysin stimulation than children with pneumococcal carriage positive children, both in peripheral blood mononuclear cells and tonsillar mononuclear cells. IL-17A may be necessary for preventing infection in our findings, however, were at odds with those of Jiang et al. who showed that Treg cells were upregulated and Th17 cells were down regulated in children in pneumococcus-positive groups' adenoids. On the other hand, our data revealed elevated IL-17A expression in the SDB patients who carried pneumococcal disease. The variations between the studies may have been influenced by various patient groups and disease contexts. We thoroughly gathered the microbiological status, immunologic pattern, and clinical data of the enrolled children in the current investigation, which were not mentioned in the other studies. We believe that our findings more accurately reflect the situation as it actually occurs in clinical practice and hold promise for the development of novel therapies that specifically target the IL-17A-mediated immune response.

OME is a common juvenile condition that frequently develops after AOM, while it can also develop without AOM18 if there is Eustachian tube dysfunction. OME that is left untreated can result in hearing loss, which can harm children's speech development, academic performance, and balance27. OME is a common juvenile condition that frequently develops after AOM, while it can also develop without AOM18 if there is Eustachian tube dysfunction. OME that is left untreated can result in hearing loss, which can harm children's speech development, academic performance, and balance.

The nasopharyngeal adenoid is a source of inflammatory mediators, a microbial reservoir, and a mechanical blockage that causes tubal dysfunction27. Furthermore, S. pneumonia is the most frequent pathogen of AOM in children5,6 and one of the major colonizers in nasopharyngeal adenoid. Determining the function of the IL-17A-mediated immune mechanism in paediatric adenoid and its relationship to pneumococcal carriage is crucial. The potential processes connected to OME and pneumococcal carriage are clarified by this study. Children with persistent OME or recurrent AOM who require repeated breathing tube insertion may benefit from innovative treatments in the future that can alter the balance of Th17/Treg function or directly target the IL-17A related inflammatory response. There were a number of restrictions that this study should take into account. First, there was no normal control group in this study, making it impossible to tell which factor is up- or down regulated. The ethical issues associated with performing an intrusive biopsy on the adenoid tissue make it challenging to find suitable and healthy children, nevertheless. Instead, we compared our results to those from earlier research using both human and animal models. Second, this study suggests a connection between IL-17A and pneumococcal carriage. Our findings did not support any linked processes or causal connections. Finally, more patient groups and large-scale research are required to fully understand the relationship between pneumococcal carriage and IL-17A activity as well as how these factors affect clinical outcomes [1-14].

CONCLUSION

We found that SDB patients who tested positive for pneumococcal carriage had considerably greater levels of adenoidal IL-17A and IL-17A:IL-10 mRNA than those who tested negative. The OME group, however, did not find these variations to be noteworthy. These findings suggested that the prolonged or chronic pneumococcal carriage in OME patients may be brought on by a minimal response to IL-17A-mediated mucosal clearance.

ISSN: 0975-8585

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