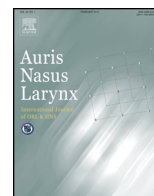




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Innate immunity gene expression by epithelial cells of upper respiratory tract in children with adenoid hypertrophy

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ABSTRACT

Background: A major role of the innate immunity in the defence of mucosal tissue is well established. However, a balance between the main components of the immunity such as toll-like receptors (TLRs) and defensins in the pathology of upper respiratory tract in children has not been addressed yet. Our aim was to investigate the gene expression of some TLRs as well as alpha and beta-defensins in children suffered from adenoid hypertrophy in comparison with healthy children.

Methods: Samples (nasal epithelium and adenoids) from patients with hypertrophic adenoids (n = 77) and control group (n = 33) were investigated. Quantification of HBD-1 and 2 mRNA, alpha-defensin-HNP1 and toll-like receptors (TLR) 2, 4 and 9 mRNA expression was performed by real-time polymerase chain reaction (PCR). The detection of TLR4 and TLR9 was performed by immunohistochemistry.

Results: The main finding of the study is a dramatic up-regulation of TLR2 and TLR4 expression (but down-regulation of TLR9) along with a significant reduction in the expression of the defensins in children with adenoid hypertrophy.

Conclusion: The data suggest that one of the mechanisms of mucosal involvement in the pathogenesis of upper respiratory tract infection might be a disbalance between TLRs and defensins revealed in our study.

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1. Introduction

Inflammatory diseases of the upper respiratory tract (URT) and middle ear mucous membranes resulting in adenoid vegetations and tonsillar hypertrophy are major issues in pediatric otorhinolaryngology [1,2]. Adenoidectomy and adenotonsilotomy are the most common surgical interventions for the treatment of this pediatric pathology, but conservative treatments are now

considered valid alternatives in clinical practice because of advancements in the understanding of the crucial immunological functions of these lympho-epithelial organs in immune system development during childhood [3]. Whether surgical or conservative treatment should be preferred is currently under debate [4]. Issues with diagnosing integrated abnormalities in children with pharyngeal tonsil hypertrophy (adenoids), which can be accompanied by blocking of nasal breathing, various forms of otitis, and hearing loss remain to be elucidated.

It is well known that the mucosa-lined surfaces of the URT, including the nasopharynx, are the first line of defense against potential pathogens [5,6]. It was recently shown that the innate immune system plays a key role in protecting the mucous membranes against various pathogens through pattern recognition receptors, including Toll-like receptors (TLRs), which detect pathogens and function as signaling molecules [7–11]. Recognition of pathogens and endogenous TLR ligands promotes the activation of appropriate genes that encode pro-inflammatory cytokines, antimicrobial peptides (PMPs), and other defense molecules [12,13]. It is known, that cytokines such as tumor necrosis factor-alpha (TNF- α), interleukin (IL)-1 β , IL-6 and IL-8 have the most pro-inflammatory activity. They play some role in the development of inflammation including chronic otitis and adenoiditis [14].

Mucosal immunity in the URT is formed in the lymphoid tissue of the nasopharynx. The epithelial cells on the surface of the nasopharynx form the interface between the host and the environment and are one of the main structures that allow for innate immunity development.

So, research, using methods of molecular genetics and immunohistochemistry, was designed as applied research to help clinicians in the choice between conservative or surgical treatment in children suffered from adenoid hypertrophy. This was an attempt to turn out why one child with adenoids required adenoidectomy but for other conservative treatment was sufficient. Obviously, that a difference in clinical course depends on the persona I reactivity resulting from the state of innate immunity of the child.

Our study aimed to analyze the expression of genes TLR2, TLR4, TLR9, HBD-1, HBD-2, HNP-1 in cells and production TNF- α , IL-1 β , IL-8 in nasal and nasopharynx secret in healthy children and in children with adenoid hypertrophy.

2. Materials and methods

This study included 122 children aged from 2 to 7 years old (male and female) suffering from adenoid hypertrophy of second or third degree (common clinical parameter estimated through the vomer length or area of the choanal openings covered by adenoids). Children were examined and treated at the Ear, Nose and Throat (ENT) department of Morozov Municipal Children's Hospital.

The criteria for inclusion in the study were the absence of viral infection, acute inflammatory diseases, or escalation of chronic diseases or allergies and a lack of adenoiditis effects one month before the study. For most of the examined children therapeutic treatment was indicated, 39 of them made up group I. Thirty-eight children had indications for surgical treatment – adenoidectomy under endoscopic visualization (group II). The control group was composed of 33 healthy children of 3–7 years of age who underwent routine examination at our hospital (healthy group) [15]. The control group included outpatients who suffered from no more than 1 or 2 acute viral infections a year.

The patient groups were compared according to complaints and clinical manifestations as follows: difficulty in nasal breathing, snoring at night, sniffles, frequency of colds, frequency of acute catarrhal or purulent middle ear infections, otitis media with effusion, and tympanometry measures.

The percentage of children who complained of difficulty in nasal breathing was significantly higher in group II than in group I. Complaints of snoring at night and sniffles were not significantly different between the studied groups.

In group II, 26 of 38 (68.4%) children had frequent colds. Over the previous 6 months of observation, an incidence of acute catarrhal otitis was observed in 28 patients (73.7%), acute purulent otitis was observed in 9 (23.7%) children, and bilateral secretory otitis media was observed in 16 children (42.1%). When we analyzed these data, it was clear that the percentage of children who had acute catarrhal otitis media or purulent otitis and the percentage of children suffering from frequent colds were higher in group II than in group I. Thus, children in group II truly needed adenoidectomy under endoscopic control. **Table 1** presents the clinical characteristics of the children in the compared groups.

Table 1

Percentage of clinical disorders in children with adenoid hypertrophy (groups I and II) and healthy children (control group).

Clinical manifestations	Patients with adenoids of degree II–III Therapeutic treatment without adenoidectomy Group I (n = 39)	Patients with adenoids of degree II–III, complicated by frequent otitis Treatment with adenoidectomy Group II (n = 38)	Healthy children Control group (n = 33)
Difficulty in nasal breathing	23 (59%)	31 (82%)	0 (0%)
Snoring at night	16 (41%)	18 (47%)	0 (0%)
Sniffles	9 (23%)	8 (21%)	0 (0%)
Frequency of colds	19 (48%)	26 (68%)	5 (15%)
Acute catarrhal	12 (31%)	28 (74%)	4 (12%)
Acute purulent otitis media	0 (0%)	9 (24%)	0 (0%)
Bilateral otitis media with effusion	8 (20%)	16 (42%)	0 (0%)
Registered tympanometry type “b” in both ears	8 (20%)	16 (42%)	0 (0%)

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