Rhinology

The effect of passive smoking on bacterial colonisation of the upper airways and selected laboratory parameters in children

L’effetto del fumo passivo sulla colonizzazione batterica delle alte vie aeree e su determinati parametri di laboratorio nei bambini

G. BUGOVA1, M. JANICKOVA2, B. UHLIAROVA3, R. BABELA4, M. JESENAK5

1 Department of Otorhinolaryngology, Head and Neck Surgery, Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, University Hospital, Martin, Slovakia; 2 Department of Stomatology and Maxillofacial Surgery, Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, Martin, Slovakia; 3 Department of Otorhinolaryngology, FD Roosevelt Faculty Hospital, Banska Bystrica, Slovakia; 4 Institute of Healthcare Disciplines, St. Elisabeth University, Bratislava, Slovakia; 5 Department of Paediatrics, Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, University Hospital, Martin, Slovakia

SUMMARY

Exposure to tobacco smoke is associated with a higher risk of respiratory tract diseases. The aim of this study was to determine the influence of passive smoking on selected characteristics of children with adenoid hypertrophy. Sixty-one children with adenoid hypertrophy were enrolled in the prospective study. Differences in bacterial colonisation of middle nasal meatus and nasopharynx and changes in selected laboratory immune and inflammatory markers according to the tobacco smoke exposure were analysed. Exposure to tobacco smoke was associated with significantly higher colonisation of pathogenic bacteria and polymicrobial growth of pathogenic bacteria (≥ 2 bacteria) in middle nasal meatus compared to non-exposed children (P = 0.045, P = 0.032, respectively). Identification of pathogenic bacteria in the middle nasal meatus did not correlate with isolation of pathogenic bacteria in the nasopharynx in either group of children. Parameters of humoral immunity in serum, IgA and IgG, were detected at higher concentrations in children exposed to tobacco smoke (P = 0.047, P = 0.031, respectively). Differences in selected parameters of cellular immunity in peripheral blood according to passive smoking were not observed. Tobacco smoke exposure is related to increased colonisation by pathogenic bacteria in middle nasal meatus and elevation of IgA and IgG in peripheral blood, but does not seem to influence markers of cellular immunity parameters in children with adenoid hypertrophy. Avoidance of passive smoking could be recommended as a universal preventive strategy against microbial colonisation of the upper airways and development of various inflammatory diseases in children, e.g. adenoid hypertrophy.

KEY WORDS: Mucosal microbiota • Passive smoking • Pathogenic bacteria • Upper airways • Immunity

RIASSUNTO

L’esposizione al fumo di sigaretta è associato ad un alto rischio di sviluppare malattie del tratto respiratorio. L’obiettivo di questo studio è stato quello di determinare l’influenza del fumo passivo su determinate caratteristiche dei bambini con ipertrofia adenoidea. Sessantuno bambini con ipertrofia adenoidea sono stati arruolati in questo studio prospettico. Sono stati analizzati differenze nella colonizzazione batterica del meato medio e del nasofaringe e cambiamenti di determinati parametri di laboratorio immunologici e di marker dell’infiammazione in relazione all’esposizione al fumo di tabacco. L’esposizione al fumo è stata associata in maniera significativa alla colonizzazione di batteri patogeni e alla crescita polimicrobica di batteri patogeni (≥ 2 batteri) nel meato nasale medio, rispetto ai bambini non esposti (P = 0,045, P = 0,032, rispettivamente). L’identificazione di batteri patogeni nel meato medio non è stata accompagnata all’isolamento di batteri patogeni nel nasofaringe di entrambi i gruppi di bambini. Parametri sierici dell’immunità umorale, quali IgA e IgG, sono risultati notevolmente più elevati nei bambini esposti (P = 0,047, P = 0,031, rispettivamente). Tuttavia non sono state trovate differenze nei parametri sierici riguardanti l’immunità cellulare. In conclusione l’esposizione al fumo di tabacco sembra essere correlata ad un incremento della colonizzazione da parte di batteri patogeni del meato medio ed ad un aumento delle IgA e delle IgG nel sangue periferico, mentre non sembra influenzare i marker dell’immunità cellulare nei bambini con ipertrofia adenoidea. Evitare il fumo passivo dovrebbe essere raccomandato come strategia preventiva universale contro la colonizzazione microbica delle alte vie aeree e lo sviluppo di svariate malattie infiammatorie dei bambini, come ad esempio l’ipertrofia adenoidea.

PAROLE CHIAVE: Microbiota • Fumo passivo • Batteri patogeni • Alte vie respiratorie • Immunità
Introduction

Exposure to environmental tobacco smoke (ETS) is associated with a variety of health effects, including cancer, cardiovascular diseases and/or respiratory illnesses. Tobacco is also a major burden to people who do not smoke. As developing individuals, children are particularly vulnerable to the negative effects of second-hand smoke (SHS). Furthermore, they are unable to influence their own degree of exposure. Worldwide, at least 40% of children are regularly exposed to SHS predisposing them to upper and lower respiratory infections as well as asthma. Moreover, long-term exposure to ETS creates a state of permanent inflammation and an imbalance in the lipid profile that leads to lipid accumulation in the blood vessels of the heart and aorta. Children with long-term exposure to ETS may have an elevated risk for the development of premature coronary artery disease. Simonetti et al. found that in healthy children, parental smoking is an independent risk factor for higher blood pressure, adding to other familial and environmental risk factors.

Mucosal microbiota of the upper airways is very important for health and diseases and its development begins suddenly after the birth. Changes in airway microbiota are associated with acute and chronic consequences. There are many positive factors influencing the correct composition of microbiota, e.g. breastfeeding. On the other hand, negative changes of mucosal microbiota may contribute to the development of pathological conditions such as sinusitis, otitis media or chronic airway inflammation. Passive smoking changes the microbial colonisation of the airways. It has been shown that nasopharyngeal microbiota is related to the frequency of upper respiratory infection and sinusitis and can be a determinant for infection spread to the lower airways. It has been suggested that commensal colonisation may interfere with pathogen colonisation. Adenoids serve as a bacterial reservoir for upper airway infections and their removal is followed by changes in upper airway microbiota and a decrease in the rate of respiratory infections.

In our prospective study, we aimed to evaluate the modifying effect of passive smoking on selected parameters in children with adenoid hypertrophy indicated for endoscopic adenotomy due to mechanic upper airway obstruction or recurrent respiratory tract infections. Moreover, we studied the effects of passive smoking on the composition of upper airway mucosal microbiota in the nasopharynx and middle nasal meatus.

Materials and methods

Design of the study

The prospective study was conducted with 61 children divided into 2 groups according to tobacco smoke exposure. All children enrolled in the study were scheduled to endoscopic adenotomy for adenoid hypertrophy (due to mechanic obstruction of upper airways or recurrent respiratory tract infections) at the Department of Otorhinolaryngology, Head and Neck Surgery, Comenius University, Jessenius Faculty of Medicine, University Hospital in Martin, Slovakia.

Children treated with systemic or local antibiotics within the 2 weeks before enrolment, recent respiratory infection, increased level of C-reactive protein and those with recurrent tonsillitis were excluded from the study. Smoking exposure was recorded according to the questionnaires. Differences in bacterial colonisation of the middle nasal meatus and nasopharynx and changes in humoral and cellular immunity according to tobacco smoke exposure were analysed. The study was approved by the Ethics Committee of Jessenius Medical Faculty, Comenius University in Martin, Slovakia (EK 1515/2014). An informed consent form was signed by all parents of participating children.

Smoking exposure

Only exposure to parental smoking was recorded as parents were considered to be the closest individuals caring for the child. The level of exposure when smokers were other than the parents (e.g. grandparent or sibling) and parent was a non-smoker could not be evaluated and those cases were excluded from the study. Exposure to smoke other than tobacco smoke was not recorded. All the children were from urban areas.

Cultivation studies

Middle nasal meatus and nasopharyngeal swab specimens were obtained under endoscopic control by using sterile cotton-wool swabs and transported in Stuart’s transport medium to the microbiological laboratory within 2 to 4 hours. The swab was inoculated on Sheep blood agar (Columbia Bio-Rad, Bratislava, Slovakia) and Chocolate agar with bacitracin disc, Mac Conkey agar (Bio-Rad, Bratislava, Slovakia), and placed into a 7% CO₂ incubator at 37°C. Plates were examined after 18 to 24 hours of incubation. The incubation was further extended to 48 hours to detect slow-growing microbes. Identification of colonies at a genus or species level was based upon typical colony morphology by subculture, Gram stain, standard
rapid tests (catalase, pyrrolidonyl aminopeptidase - PYR and oxidase tests), identification by latex agglutination tests and biochemical tests. All pathogenic strains were tested for their susceptibility to antimicrobial agents using the agar diffusion method (by EUCAST) with commercial discs (Oxoid).

**Examination of immune parameters in peripheral blood**

Various parameters and markers of cellular and humoral immunity were evaluated. Venous blood samples were drawn from a peripheral arm vein, collected into an evacuated tube and treated with either EDTA or sodium heparin. The sampling was performed on the day before surgery. White and red components of the blood count were examined by sampling with an 18-parameter haematological ANALYSER Beyer Advia 60 (Siemens AG, Munich, Germany) according to the manufacturer’s reagents. Differential counts of leukocytes, as well as subpopulations of lymphocytes and NK cells, were assessed immediately after sampling. Cells were counted on the flow cytometer FC500 (Beckmann Coulter, Brea, CA, USA) after staining with monoclonal antibodies (Immunotech, Prague, Czech Republic) according to the manufacturer’s instructions. A four-colour fluorescent protocol was used with the following composition: CD4+CD19-FITC/CD8+CD16+CD56PE/CD3-PC5/CD45PC7. The absolute counts of all cell populations were calculated from the examined blood count. The following populations, based on the CD45+ leukocyte gate, were quantified from the CD4 vs. side scatter (SS) cytogram: lymphocytes (low SS), T lymphocytes (CD3+), T helpers (CD3+CD4+), T cytotoxic cells (CD3+8+), B lymphocytes (CD19+) and NK cells (CD3-8+16+56+). Absolute and relative counts of NK cells were also examined on the cytometer using separate analysis parameters (CD8-FITC/CD16/CD56PE/CD3PC5/CD45PC7). Serum four immunoglobulin isotypes (IgG, IgA, IgM, total IgE) were also examined.

**Statistical analysis**

Frequencies of categorical data were tabulated and evaluated with a chi-square test using Yates’s correction. For other data, median and interquartile range was calculated and tested with the Kruskal-Wallis or Mann-Whitney tests. The statistical analysis was performed with STATISTICA Cz 10. All conclusions were based on a significance level of $P < 0.05$.

**Results**

Among the 61 children in this study, 23 (37%) were exposed to SHS (14 male, 9 female, mean age 5.5 ± 3 years, range 2-16). Thirty-eight (63%) children had non-smoking parents (29 male, 9 female, aged 5.2 ± 2.8 years, range 2-16). Bacterial growth was present in 50 of the 61 samples (82%) from the middle nasal meatus and 60 of the 61 samples (98%) from the nasopharynx. Microorganisms isolated from the middle nasal meatus and nasopharynx are presented in Tables I and II. Coagulase-negative *Staphylococcus species*, *Corynebacterium species*, *Streptococcus viridans* and *Neisseria species* are considered as commensals of the upper respiratory tract. Other species of identified bacteria in our study were considered as pathogens. The pathogenic bacteria in our patients were present in 29 samples (58%) in the middle nasal meatus and 43 (72%) in the nasopharynx ($P = 0.133$). We found significantly more intense colonisation by pathogenic bacteria in the middle nasal meatus in children exposed to SHS than in children with non-smoking parents ($P = 0.045$). Polymicrobial growth of pathogens (defined as detection of at least 2 or more pathogens from one sampling site) in the middle nasal meatus was also significantly increased in children exposed to SHS ($P = 0.032$) (Fig. 1A). In the nasopharynx, there were no significant differences in the presence of commensals, pathogenic bacteria, or polymicrobial growth between children exposed and not exposed to tobacco smoke (Fig. 1B). In children exposed to SHS, *Streptococcus pneumoniae* was significantly more often isolated from the middle nasal meatus and nasopharynx ($P = 0.031, P = 0.004$, respectively). Other pathogens, such as *Staphylococcus aureus*, *Haemophilus influenzae* and *Moraxella catarrhalis*, were more often colonised in the middle nasal meatus in children exposed to SHS ($P = 0.031, P = 0.001, P = 0.05$, respectively) (Table I). Those differences were not observed in the nasopharynx. Identification of pathogenic bacteria in the middle nasal meatus did not correlate with isolation of pathogenic bacteria in the nasopharynx in either group of children. Gram-negative pathogens were isolated significantly more often from the middle nasal meatus in children exposed to SHS than in children with non-smoking parents ($P = 0.018$). There were no significant differences in the presence of gram-positive and gram-negative bacteria in the nasopharynx between children exposed and not exposed to tobacco smoke ($P = 0.723$).

Analysing the differences in selected immunological parameters according to the exposure to tobacco smoke, a possible association with passive smoking was found. Significant higher levels of IgA and IgG in peripheral blood were detected in children exposed to SHS compared to children whose parents were non-smokers ($P = 0.047, P = 0.031$, respectively). Differences in the markers of cellular immunity were not found (Table III).
Exposure to tobacco smoke has many harmful effects on the health status of exposed children. In our prospective study in a group of children with adenoid hypertrophy, we analysed the influence of passive smoking on mucosal microbiota and selected laboratory parameters. We were able to detect various significant changes and differences associated with exposure to tobacco smoke. Passive smoking was associated with increased colonisation with pathogenic bacteria and polymicrobial growth in the upper airways. Due to chronic stimulation of mucosal immunity, children exposed to tobacco smoke had higher serum levels of IgG and IgA, although no changes were observed in the cellular part of immunity.

Second-hand tobacco smoke consists of exhaled smoke...
as well as side-stream smoke that is released from the burning cigarette between inhalations, which has a very similar composition. In children, exposure to smoking is associated with upper and lower respiratory tract diseases, such as acute otitis media, asthma, wheezing, cough, bronchitis, pneumonia and impaired pulmonary function \(^\text{12}\). Both smoking and exposure to tobacco smoke in the household are associated with carriage of bacteria, such as *Neisseria meningitidis* \(^\text{13}\). In children, the intensity of exposure to environmental smoking correlates with

---

**Table III.** Differences in humoral and cellular immunity according to the second hand smoke exposure. Data are shown as mean ± SD.

| Immune parameter                  | SHS exposed  | SHS non-exposed | P      |
|-----------------------------------|--------------|-----------------|--------|
| IgG [g/L]                         | 9.87 ± 2.56  | 8.43 ± 1.79     | 0.031  |
| IgA [g/L]                         | 1.13 ± 0.39  | 0.86 ± 0.46     | 0.047  |
| IgM [g/L]                         | 0.95 ± 0.38  | 0.89 ± 0.38     | 0.477  |
| C3 [g/L]                          | 1.16 ± 0.19  | 1.16 ± 0.22     | 0.952  |
| C4 [g/L]                          | 0.25 ± 0.13  | 0.21 ± 0.07     | 0.249  |
| IgE [ IU/L]                       | 58.94 ± 76.73| 32.09 ± 29.63   | 0.566  |
| Leucocytes [10^6/L]               | 7.77 ± 1.46  | 7.80 ± 2.82     | 0.340  |
| Neutrophils [10^6/L]              | 3.15 ± 1.08  | 3.04 ± 0.83     | 0.979  |
| Lymphocytes [10^6/L]              | 3.66 ± 0.84  | 3.84 ± 2.15     | 0.548  |
| Monocytes [10^6/L]                | 0.65 ± 0.19  | 0.70 ± 0.27     | 0.603  |
| Eosinophils [10^6/L]              | 0.39 ± 0.36  | 0.25 ± 0.15     | 0.566  |
| Basophils [10^6/L]                | 0.05 ± 0.05  | 0.05 ± 0.05     | 0.862  |
| CD3^+ T lymphocytes [10^6/L]      | 2508.77 ± 701.03| 2589.93 ± 146.70| 0.430  |
| CD19^+ B lymphocytes [10^6/L]     | 627.38 ± 185.84| 656.72 ± 345.82| 0.786  |
| CD3^+CD4^+ T lymphocytes [10^6/L]| 1330.46 ± 377.87| 1374.03 ± 645.44| 0.596  |
| CD3^+CD8^+ T lymphocytes [10^6/L]| 1026.69 ± 399.34| 990.52 ± 694.42| 0.169  |
| CD15^+CD56^+ NK cells [10^6/L]    | 462.69 ± 242.76| 527.66 ± 438.57| 0.849  |
| CD4:CD8 ratio                     | 1.39 ± 0.32  | 1.48 ± 0.47     | 0.751  |

Ig: immunoglobulin; C3 and C4: parts of complement system; NK cells: natural killer cells.
respiratory infection rates, especially if the parent smokes in the same room as the child 14. In young children, *Streptococcus pneumoniae, Haemophilus influenzae* and *Moraxella catarrhalis* are the most common bacterial pathogens that cause respiratory infections, such as acute otitis media and pneumonia, as well as invasive infections, such as bacteraemia and meningitis 7. Adenoid hypertrophy is a common pathological condition in early childhood and is frequently associated with upper respiratory tract obstruction (leading to e.g. obstructive sleep apnoea syndrome) 15 and recurrent respiratory tract infections. Based on our results, passive smoking had a negative influence on selected parameters in children with adenoid hypertrophy, and could therefore represent an avoidable factor that can complicate the clinical status of these children.

Colonisation of organisms is the first step toward development of respiratory diseases. Therefore, recognition of risk factors for colonisation is important. However, data are sparse with regards to the influence of smoking on rates of nasal and nasopharyngeal colonisation with these organisms in children and adults. In the present study, we demonstrated that children exposed to smoking by parents had a significantly higher rate of pathogenic bacteria (*Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis* and *Staphylococcus aureus*) carriage than did children who were not exposed to tobacco smoke. The increased carriage rate in individuals exposed to tobacco smoke was seen in the middle nasal meatus, but not in nasopharyngeal specimens. The differences between these two sites were found also by Rawling et al. (2013) 16. Increased nasal colonisation by pathogenic bacteria may be a predisposing factor to additional spread, resulting in lower respiratory tract infections. This could be further increased during coinfection with respiratory viruses, such as influenza viruses, in children 17. In a recent study, it was shown that colonisation with *Staphylococcus* may be also beneficial to some extent, since it counteracts otopathogens 10. Despite the fact that we did not study the effect of bacterial colonisation and passive smoking on the frequency of respiratory infections, our findings might explain previous reports demonstrating that children exposed to environmental tobacco smoke more frequently experience respiratory infections, such as acute otitis media and pneumonia 7 14. Increased polymicrobial growth of pathogens in the upper respiratory tract in children exposed to SHS compared with children born to non-smoking parents found in our study also confirmed the role of tobacco smoke in the pathogenesis of respiratory tract infections in those children in early childhood.

The mechanism by which smoking and passive exposure to tobacco smoke is associated with carriage of potentially pathogenic bacteria is not fully understood. One of the possible explanations is based on mucociliary transport alterations. Several studies have shown that cigarette smoke significantly reduces the ciliary beat of respiratory epithelial cells both *in vitro* 18 and *in vivo* 19. Moreover, Tamashiro et al. (2009) found that exposure of tobacco smoke impaired ciliogenesis in a dose-dependent manner in murine sinonasal epithelial cell culture 20. Cigarette smoking is also associated with profound changes in the mechanisms of mucous production. Chronic exposure to tobacco smoke causes respiratory epithelium metaplasia with increased number and size of goblet cells and, consequently, increased mucous secretion in the upper respiratory tract. Furthermore, tobacco smoke also inhibits interleukin 8 and human β-defensin in sinonasal epithelial cell cultures derived from patients with CRS 21. These findings suggest that cigarette smoke may have a suppressive function on sinonasal innate immunity. Taken together, more intense colonisation of upper respiratory tract by pathogenic bacteria might be a result of impaired local defence mechanisms of respiratory mucosa. Moreover, respiratory pathogens (e.g. *Streptococcus pneumoniae*) are associated with increased adherence to respiratory epithelial cells in chronic exposure to cigarette smoke 22.

This study also demonstrated that gram-negative bacteria were significantly more often isolated from the middle nasal meatus in children exposed to SHS compared to non-exposed children. Similar to our results, Ertel et al. (1991) showed that the respiratory system of adult smokers is preferentially colonised by gram-negative bacilli. This is explained by better resistance of gram-negative bacteria to cigarette smoke compared to gram-positive ones 23.

On the other hand, only *Streptococcus pneumoniae* was found more often in the nasopharynx in children exposed to SHS compared to non-exposed children. Differences in other pathogens according to tobacco exposure were not observed. One explanation for this finding is the study population. All children in the present study underwent surgical treatment due to adenoid hypertrophy. Pathogens such as *Haemophilus influenzae, Streptococcus pneumoniae* and *Moraxella catarrhalis* are most commonly detected bacteria in adenoid tissue 24. Therefore, we assume that colonisation by pathogens was up-regulated due to adenoid hypertrophy, and thus the possible differences between tobacco exposure and pathogens in nasopharynx in children with adenoid hypertrophy could not be manifested.

In our study, we observed an effect of passive smoking on markers of systemic humoral immunity (IgG and IgA), which were increased in children regularly exposed to tobacco smoke compared to non-exposed children. There
are only a few reports on the effects of passive and active smoking on humoral mucosal and systemic immunity in the literature. It has been shown that smokers may have increased 25 but also decreased concentrations of IgA in saliva 26. Exposure of cigarette smoke supports the activation of innate immunity (e.g. Toll-like receptors, neutrophils) with possible effects on parameters of adaptive humoral immunity 27. On the other hand, tobacco smoke increases the susceptibility of respiratory mucosa to various viruses and bacteria 28, which can contribute to the recurrent respiratory infections, chronic stimulation of mucosal immune system and its hypertrophy. Hypertrophic lymphoid tissue in the upper airways is associated with increased levels of saliva IgA 29. Maternal smoking increases chronic upper respiratory symptoms and saliva IgA levels in children 30. The increased levels of IgA and IgG in peripheral blood observed in our children might be therefore attributed to the chronic stimulation of lymphoid tissue of upper airways due to recurrent respiratory infections, which can be the result of exposure to passive smoke. Moreover, this activation of humoral immunity can also be supported by chronic inflammation induced by exposure to tobacco smoke. Passive smoking was also shown to be a risk factor for the development of adenoid hypertrophy in another study 31. Recently, it was shown that adenoid tissue is related to a Th-2 deviated immune response. This could aggravate the harmful effect of passive smoking on mucosal immunity and microbial colonization 32. It was further shown that smoking induces nasopharyngeal lymphoid hyperplasia, and therefore passive smoking could also be considered as a risk factor for development of adenoid hypertrophy 33. Another important contributing mechanism of passive smoking is the induction of persistent oxidative stress and endothelial dysfunction 34.

Conclusions

Tobacco smoke exposure is related to increased colonisation by pathogenic bacteria in the middle nasal meatus, but not in the nasopharynx. Identification of bacteria from the middle nasal meatus did not correlate with isolation of pathogenic bacteria from the nasopharynx. The upper respiratory tract is preferentially colonised by gram-negative bacteria in children exposed to second hand smoke. Chronic passive smoking alters the composition of upper airway mucosal microbiota and thus contributes to the development of several pathological conditions. Exposure to tobacco smoke leads to elevation of IgA and IgG in peripheral blood, but does not influence markers of cellular immunity in children. Therefore, it can be suggested that avoidance of passive smoking represents a universal strategy for prevention of different inflammatory conditions in children and could be recommended as a standard part of complex management of children with recurrent respiratory tract infections and adenoid hypertrophy.

Acknowledgements

The study was supported by Grant from the Ministry of Education, Science, Research and Sport of the Slovak Republic VEGA 1/0252/14 and by the project Measurement of Kinetics of Cilia in Respiratory Tract (ITMS 26220220019), co-financed by EU sources.

Conflict of interest statement

None declared.

References

1. Oberg M, Jaakkola MS, Woodward A, et al. Worldwide burden of disease from exposure to second-hand smoke: a retrospective analysis of data from 192 countries. Lancet 2011;377:139-46.
2. Banovcin P, Seidenberg J, Von der Hardt H. Assessment of tidal breathing patterns for monitoring of bronchial obstruction in infants. Pediatr Res 1995;38:218-20.
3. Yuan H, Wong LS, Bhattacharya M, et al. The effects of second-hand smoke on biological processes important in atherogenesis. BMC Cardiovasc Disord 2007;7:1.
4. Simonetti GD, Schwartz R, Klett M, et al. Determinants of blood pressure in preschool children: the role of parental smoking. Circulation 2011;123:292-8.
5. Brisbroek G, Bosch AATM, Wang X, et al. The impact of breastfeeding on nasopharyngeal microbial communities in infants. Am J Respir Crit Care Med 2014;190:298-308.
6. Santee CA, Nagalingam NA, Faruqi AA, et al. Nasopharyngeal microbiota composition of children is related to the frequency of upper respiratory infection and acute sinusitis. Microbiome 2016;4:34.
7. Greenberg D, Givon-Lavi N, Broides A, et al. The contribution of smoking and exposure to tobacco smoke to streptococcus pneumoniae and haemophilus influenzae carriage in children and their mothers. Clin Infect Dis 2006;42:897-903.
8. Sakwinska O, Schmid VB, Berger B, et al. Nasopharyngeal microbiota in healthy children and pneumonia patients. J Clin Microbiol 2014;52:1590-4.
9. Teo SM, Mok D, Pham K et al. The infant nasopharyngeal microbiome impacts severity of lower respiratory infection and risk of asthma development. Cell Host Microbe 2015;17:704-15.
10. Chonmaitree T, Jennings K, Golovko G, et al. Nasopharyngeal microbiota in infants and changes during viral upper respiratory tract infection and acute otitis media. PLoS One 2017;12:e0180630.
11 Subtil J, Rodrigues JC, Reis L, et al. Adenoid bacterial colonization in a paediatric population. Eur Arch Otorhinolaryngol 2017;274:1933-8.

12 Joya X, Manzano C, Álvarez AT, et al. Transgenerational exposure to environmental tobacco smoke. Int J Environ Res Public Health 2014;11:7261-74.

13 Stuart JM, Cartwright KA, Robinson PM, et al. Effect of smoking on meningococcal carriage. Lancet 1989;2:723-5.

14 Blizzard L, Ponsonby AL, Dwyer T, et al. Parental smoking and infant respiratory infection: how important is not smoking in the same room with the baby? Am J Public Health 2003;93:482-8.

15 Sujanska A, Durdik P, Rabasco J, et al. Surgical and nonsurgical therapy of obstructive sleep apnea syndrome in children. Acta Medica (Hradec Kralove) 2014;57:135-41.

16 Rawlings BA, Higgins TS, Han JK. Bacterial pathogen in the nasopharynx, nasal cavity, and osteomeatal complex during wellness and viral infection. Am J Rhinol Allergy 2013;27:39-42.

17 Wadowsky RM, Mietzner SM, Skoner DP, et al. Effect of experimental influenza A virus infection on isolation of Streptococcus pneumoniae and other aerobic bacteria from the oropharynxes of allergic and nonallergic adult subjects. Infect Immun 1995;63:1153-7.

18 Cohen NA, Zhang S, Sharp DB, et al. Cigarette smoke condensate inhibits transepithelial chloride transport and ciliary beat frequency. Laryngoscope 2009;119:2269-74.

19 Karaman M, Tek A. Deleterious effect of smoking and nasal septal deviation on mucociliary clearance and improvement after septoplasty. Am J Rhinol Allergy 2009;23:2-7.

20 Tamashiro E, Xiong G, Anselmo-Lima WT, et al. Cigarette smoke exposure impairs respiratory epithelial ciliogenesis. Am J Rhinol Allergy 2009;23:117-22.

21 Lee WK, Ramanathan M, Spannhake EW, et al. The cigarette smoke component acrolein inhibits expression of the innate immune components IL-8 and human beta-defensin 2 by sinonasal epithelial cells. Am J Rhinol 2008;21:658-63.

22 Ozlu T, Celik I, Oztuna F, et al. Streptococcus pneumoniae adherence in rats under different degrees and durations of cigarette smoke. Respiration 2008;75:339-44.

23 Ertel A, Eng R, Smith SM. The differential effect of cigarette smoke on the growth of bacteria found in humans. Chest 1991;100:628-30.

24 Marzouk H, Aynehchi B, Thakkar P, et al. The utility of nasopharyngeal culture in the management of chronic adenoiditis. Int J Pediatr Otorhinolaryngol 2012;76:1413-5.

25 Norhagen G, Engstrom PE. Effects of tobacco smoking on salivary immunoglobulin levels in immunodeficiency. Eur J Oral Sci 1998;106:986-91.

26 Doni BR, Patil S, Peerapur BV, et al. Estimation and comparison of salivary immunoglobulin A levels in tobacco chewers, tobacco smokers and normal subjects. Oral Health Dent Manag 2013;2:105-11.

27 Pace E, Ferraro M, Di Vincenzo S, et al. Oxidative stress and innate immunity responses in cigarette smoke stimulated nasal epithelial cells. Toxicol In Vitro 2014;28:292-9.

28 Zhang W, Case S, Bowler RP, et al. Cigarette smoke modulates PGE2 and host defence against Moraxella catarrhalis infection in human airway epithelial cells. Respirology 2011;16:508-16.

29 Childers NK, Powell WD, Tong G, et al. Human salivary immunoglobulin and antigen-specific antibody activity after tonsillectomy. Oral Microbiol Immunol 2001;16:265-9.

30 Noakes P, Taylor A, Hale J, et al. The effects of maternal smoking on early mucosal immunity and sensitization at 12 months of age. Pediatr Allergy Immunol 2007;18:118-27.

31 Evcimik MF, Dogru M, Cirik AA, et al. Adenoid hypertrophy in children with allergic disease and influential factors. Int J Pediatr Otorhinolaryngol 2015;79:694-7.

32 Mandapathil M, Beier UH, Graefe H, et al. Differential chemokine expression patterns in tonsillar disease. Acta Otorhinolaryngol Ital 2018;38:316-22.

33 Finkelstein Y, Malik Z, Kopolovic J, et al. Characterization of smoking-induced nasopharyngeal lymphoid hyperplasia. Laryngoscope 1997;107:1635-42.

34 Loffredo L, Zicari AM, Occasi F, et al. Passive smoking exacerbated nicotinamide-adenine dinucleotide phosphate oxidase isoform 2-induced oxidative stress and arterial dysfunction in children with persistent allergic rhinitis. J Pediatr 2018 Aug 29. pii: S0022-3476(18)30878-3. doi: 10.1016/j.jpeds.2018.06.053. [Epub ahead of print].