ORIGINAL ARTICLE

SPECIFIC FEATURES OF THE ORAL MICROBIOME IN YOUNG CHILDREN WITH LARYNGOPHARYNGEAL REFLUX AND ITS ROLE THE DEVELOPMENT OF RECURRENT RESPIRATORY DISEASES

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ABSTRACT

The aim: To examine the composition of the oral microbiome in young children with laryngopharyngeal reflux (LPR) and its role the development of recurrent respiratory diseases.

Materials and methods: There were examined 38 children with physiological gastroesophageal reflux (GER), 18 children with LPR who had a medical history of recurrent bronchitis and 17 healthy children (control group). The study included the collection of anamnesis, objective examination. The qualitative and quantitative microbial composition of the upper respiratory tract was performed obtained by oropharyngeal deep swab. Salivary pepsin level and IL-8 were determined by enzyme-linked immunosorbent assay.

Results: This research showed significant alterations in the oral microbiome of patients with GER and LPR as compared to healthy control. We found that gram-negative microbiota such as *Klebsiella pneumoniae, Escherichia coli, Proteus vulgaris*, *Proteus spp.* and *Candida albicans* were identified in children with GER and LPR compared to the healthy control. At the same time, the amount of such a representative of the normal microbiome as *Streptococcus viridans* in children with LPR was sharply reduced. There were established a much higher mean salivary pepsin level of the patients with LPR than in the GER and control group. We found the association between high pepsin levels, saliva IL-8 levels and frequency of respiratory pathology in children with LPR.

Conclusions: Our study confirms that increased levels of pepsin in saliva are a risk factor for recurrent respiratory diseases in children with LPR.

KEY WORDS: GER, LPR, oral microbiome, children

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INTRODUCTION

Gastroesophageal reflux (GER) is a common and normal physiological process in children whatever their age. Gastroesophageal reflux is generally defined as retrograde passage of gastric contents into the esophagus with or without regurgitation/vomiting [1]. Laryngopharyngeal reflux (LPR) is the result of the reflux of gastric contents into the laryngopharynx mucosa [2]. LPR can lead to upper respiratory tract pathologies via three mechanisms. The most important mechanism is a direct noxious effect of gastric contents on the mucosa, causing its swelling, mucus hypersecretion, ciliary dyskinesia, and stimulation of the secretion of inflammatory mediators. Another mechanism consists of triggering a vagus nerve response supported by excessive vagal reactivity, which is observed in patients with LPR compared with healthy people. The third hypothesis postulates an association between LPR with changes of oral microbiome [3, 4].

Several studies show that acid does not damage the mucosa by itself. These studies state that pepsin plays the key role in mucosal injury, which can explain the mechanism of injury in the laryngeal mucosa in non-acidic reflux [5]. Pepsin is considered to be the most aggressive protease in the gastroduodenal refluxate. Pepsin is undetectable in the laryngeal mucosa of healthy individuals [6]. Pepsin is refluxed to the extraesophageal areas where it adheres to the epithelium [7]. Pepsin can be found in many different tissue samples such as laryngeal mucosa, paranasal sinus mucosa, saliva, middle ear effusion, tracheal secretions and bronchoalveolar lavage fluid [8]. Pepsin remains active up to pH 6.5. Then, it is inactivated but is still stable and can be reactivated if the pH drops. The most recent studies show that pepsin can also be reactivated within the acidic intracellular environment after receptor mediated uptake of pepsin by laryngeal epithelial cells, even if the pH in the throat is up to 7.4.

Moreover, the laryngeal epithelial cells are susceptible to pepsin even in a non-acidic environment because pepsin stimulates the expression of many proinflammatory cytokines and receptors, such as IL6, IL8, TNF-α and others [4, 9].

IL-8 is a multifunctional cytokine that participates in in both acute inflammation and chronic inflammatory injury associated with LPR [10]. The main function of IL-8 is to attract neutrophils and activate them. Activation of neutrophils by IL-8 results in development of enzymes involved in tissue degeneration and development of lesions. IL-8 plays an important role in immunity of the oral cavity [11].

The saliva is the main regulator of the total number of microorganisms in the oral cavity. Changes in its physical and chemical properties as a result of LPR may contribute to oral dysbiosis [2]. Poor oral health has long been recognized as a clinical risk factor for developing lung infections. There are no data about influence of LPR on the composition of the oral microbiome in young children and its role the development of recurrent respiratory pathology.

THE AIM

The aim of this study was to examine the composition of the oral microbiome in young children with LPR and its role the development of recurrent respiratory diseases.

MATERIALS AND METHODS

The study was pilot in nature, and the article discusses results obtained during the pilot stage.

In accordance with the aim of the study, we performed a clinical and laboratory examination of 73 children aged from 3 months to 7 years, who were subsequently subdivided into the 2 study groups. First group included thirty-eight children with physiological GER (mean age - 6.8 ± 0.9 months). Second group included eighteen children with LPR who had a medical history of recurrent bronchitis (mean age - 4.6±0.04 years). Seventeen clinically healthy children (mean age – 5.7±0.3 months) constituted the control group. Signed informed consent was obtained from all participants before the study.

All the study subjects were screened for the criteria of exclusion: acute inflammatory diseases of the gastrointestinal tract, its congenital pathology (pylorostenosis, esophageal atresia, congenital diaphragmatic hernia), chronic hereditary and congenital bronchopulmonary diseases (bronchial asthma, cystic fibrosis, primary ciliary dyskinesia, congenital malformations of bronchus and lungs), severe organic lesions of the central nervous system, accompanied by dysphagia, children had taken antibiotics in the past 1 month.

Anamnestic data for each study subject were collected using a semi-structured questionnaire and interviews with parents, as well as through analysis of the children's medical records. Clinical-anamnestic criteria and laboratory-instrumental methods (including fibroesophagogastroscopy) were used for diagnostic GER.

Salivary pepsin levels were determined for the diagnosis of LPR. All parents were instructed to provide three saliva samples of 1 ml volume: in children of the 1st group – the first on waking prior to eating, immediately after regurgitation and 1 hour after the meal; in children of the 2nd and control group – the first on waking prior to eating, half an hour before the meal and 1 hour after the meal. We used an enzyme-linked immunosorbent assay (Human Pepsin ELISA Kit, Elabscience, USA) for quantitative determination of the studied salivary pepsin levels. The sensitivity of the method is 37.50 pg/ml, the detection range – 62.50-4000 pg/ml. Specificity of the method: this kit recognizes human PP in samples.

Salivary IL-8 was measured by enzyme-linked immunosorbent assay kit (Human IL-8 ELISA Kit, Elabscience, USA). The sensitivity of the method is 4.69 pg/mL, the detection range – 7.81-500 pg/ml.

The study of qualitative and quantitative microbial composition of the upper respiratory tract was performed obtained by oropharyngeal deep swab according to the standard method. The collection of biomaterials was carried out in the morning on an empty stomach, after using the toilet in the oral cavity, into a disposable sterile sealed container. Clinical samples were delivered to the laboratory within an hour after the sampling of the material and inoculated on ready-made nutrient media made at the factory.

Statistical data processing was performed using standard statistical analysis software Statistica for Windows v. 6.1. Shapiro-Wilks test for normality was run to evaluate the distribution of quantitative variables. Numerical data were expressed as medians (Me) and the interquartile range (IQR, [Q25; Q75]) or as mean ± standard error according to their parametric distribution. The Mann-Whitney U-test was used to evaluate the differences between the independent groups for quantitative values, and Pearson's χ^2 test was run to compare the qualitative characteristics in the study groups. Statistical significance was defined as $p<0.05$.

This study was conducted according to the declaration of Helsinki on Biomedical Research Involving Human Subjects. Ethical approval for the research protocol was granted by the Commission on Biomedical Ethics of the Dnipro State Medical University.

RESULTS

The GER ang LPR occurrence in boys and girls was similar. There were 52.6 % of girls and 47.4 % of boys in 1st group and 44.4 % of girls and 55.6 % of boys in 2nd group.

The regurgitation incidence in children of the 1st group was more often before 3 months of age (73.7 % vs. 44.4 %, $\chi^2 = 4.55$; $p<0.05$), and in children of the 2nd group – after 6 months of age (33.3 % vs. 5.3 %, $\chi^2 = 7.86$; $p<0.01$) (Table I).

Children of the 2nd group were more likely to be born prematurely than children of the 1st group (55.6 % vs. 21.1 %, $\chi^2 = 6.67$; OR=4.7 [1.4-15.8]; $p<0.05$) and more often were artificial feeding in the first year of life (66.7

Table I. Clinical and anamnestic characteristics of children of 1st and 2 nd groups, n (%)

Characteristics	1 group (n=38)	2 group (n=18)	χ^2	p	OR [95%CI]
Age, M±m	6.8 ± 0.9 months	4.6 ± 0.04 years			
Threatened abortion	14 (36.8)	5 (27.8)	0.45	p>0.05	0.7 [0.2-2.2]
Toxicosis	16 (42.1)	7 (38.9)	0.05	p>0.05	0.9 [0.3-2.8]
Urogenital pathology in mother	4 (10.5)	2 (11.1)	0.00	p>0.05	1.1 [0.2-6.4]
Fetal hypoxia	6 (15.8)	4 (22.2)	0.34	p>0.05	1.5 [0.4-6.3]
Delivery was:					
natural	20 (52.6)	8 (44.4)	0.33	p>0.05	0.7 [0.2-2.2]
C-section	18 (47.4)	10 (55.6)	0.33	p>0.05	1.4 [0.5-4.3]
Child was born:					
full-term	26 (68.4)	8 (44.4)	2.94	p>0.05	0.4 [0.1-1.2]
preterm	8 (21.1)	10 (55.6)	6.67	p<0.05	4.7 [1.4-15.8]
post-term	4 (10.5)	-	2.04	p>0.05	-
Feeding was:					
natural	8 (21.1)	2 (11.1)	0.82	p>0.05	0.5 [0.1-2.5]
artificial	14 (36.8)	12 (66.7)	4.37	p<0.05	3.4 [1.1-11.2]
partial breastfeeding	16 (42.1)	4 (22.2)	2.10	p>0.05	0.4 [0.1-1.4]
Violation of the feeding technique	16 (66.7)	3 (16.7)	11.79	p<0.01	0.3 [0.1-1.1]
Regurgitation starts:					
up to 3 months	28 (73.6)	6 (33.3)	8.34	p<0.01	0.2 [0.1-0.6]
from 3 to 6 months	8 (21.1)	4 (22.2)	0.01	p>0.05	1.1 [0.3-4.2]
after 6 months	2 (5.3)	8 (44.5)	12.78	p<0.01	14.4 [2.6-78.9]
Family history of GERD	8 (21.1)	10 (55.6)	6.67	p<0.05	4.7 [1.4-15.8]
Acute laryngitis	5 (13.2)	7 (38.9)	4.80	p<0.05	4.2 [1.1-16.0]
Wheezing during 1 year of life	7 (18.4)	11 (61.1)	10.21	p<0.01	7.0 [2.0-24.4]

Table II. Oral microbiota in children of 1st, 2nd and control group

Oral microbiota	1 group (n=38)	Control (n=17)	p level	2 group (n=18)	Control (n=17)	p level
Gram-positive, n (%)	22 (57.9)	17 (100.0)	p<0.01	16 (88.9)	17 (100.0)	p>0.05
Gram-negative, n (%)	10 (26.3)	0.0	p<0.05	8 (44.5)	0.0	p<0.01
Mixed, n (%)	8 (21.1)	0.0	p<0.05	10 (55.6)	0.0	p<0.001

Table III. Salivary pepsin values of the two groups

Pepsin, pg/ml	1st group (n=38)	2nd group (n=18)	Control (n=17)
M±m	456.8±56.9*	672.0±60.6**	28.5±11.6
Min-Max	139.4-1183.8	432.6-1467.5	0.0-141.7
Me	398.1	560.4	0.0
25-75%	255.3-608.3	514.0-863.9	0.0-28.7

* - difference between 1st and control group, $p<0.05$

** - difference between 2nd and control group, $p<0.05$

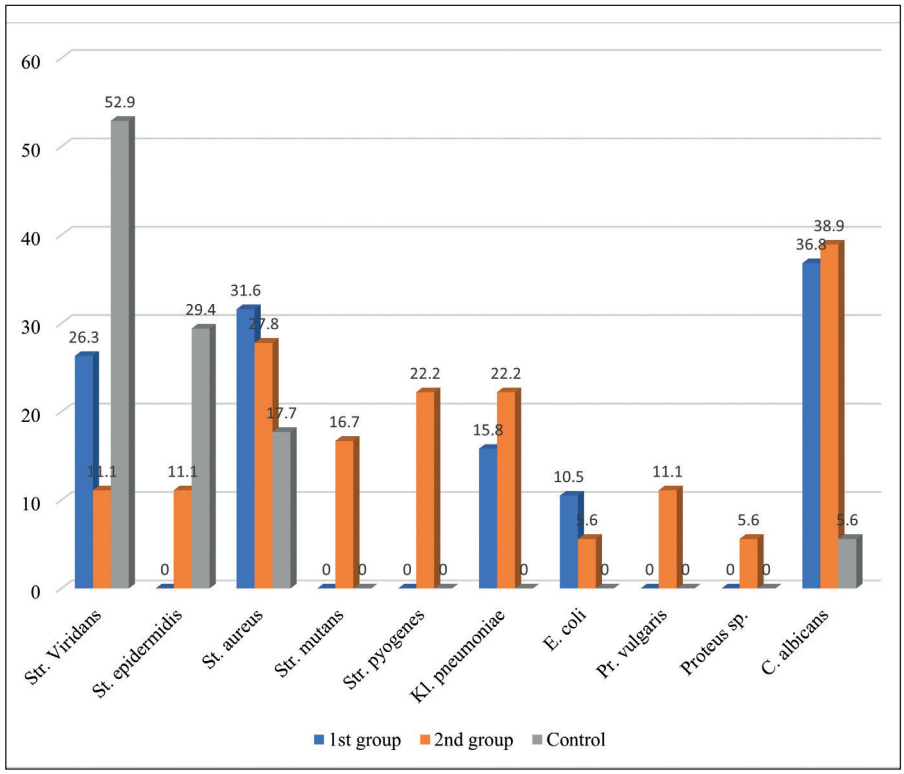

Fig. 1. Oral microbiome in children of 1st, 2nd and control group.

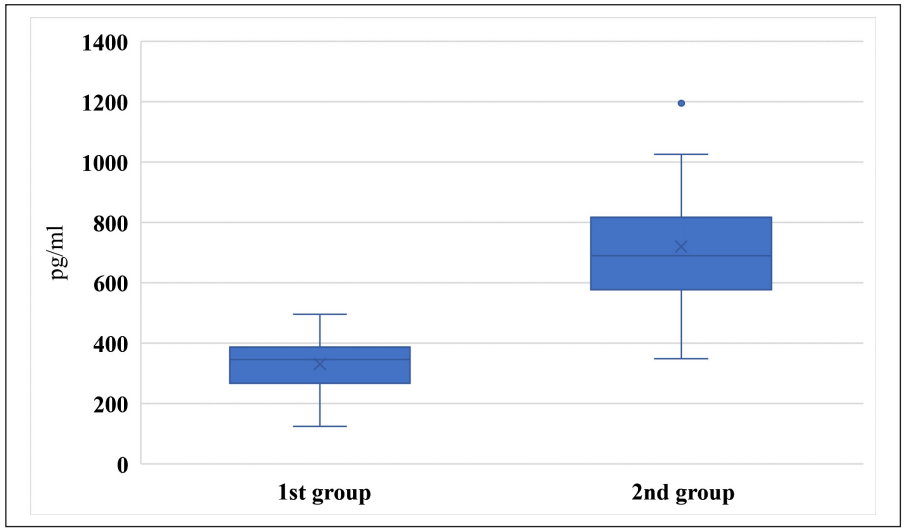

Fig. 2. The average level of saliva IL-8 in patients of the 1st and 2nd groups

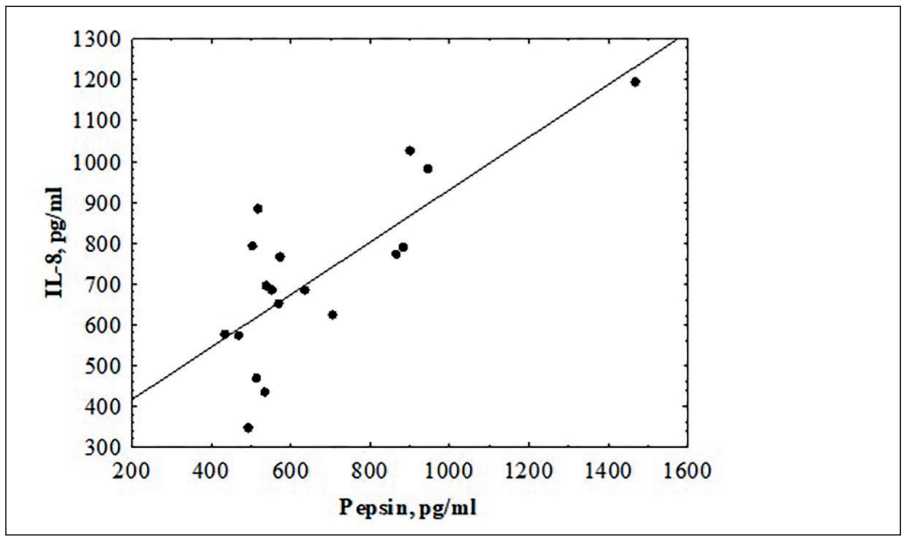

Fig. 3. Correlation between pepsin level and saliva IL-8 in children of 2nd group

% vs. 36.8 %, $\chi^2 = 4.37$; OR=3.4 [1.1-11.2]; $p<0.05$). No significant correlations were found between the severity of the regurgitation incidence and the nature of breastfeeding in both the 1st and 2nd groups.

Violations in feeding technique were more common in children of the 1st group (66.7 % vs. 16.7 %, $\chi^2 = 11.79$; $p<0.01$), among them the most common was aerophagia - in 33.3 %, as well as feeding the child in a supine position - at 16.7 %.

Family history of GERD was observed significantly more often in children of the 2nd group (55.6 % vs. 21.1 %, $\chi^2 = 6.67$; OR=4.7 [1.4-15.8]; $p<0.05$).

Respiratory pathology during the first year of life occurred more often in children of the 2nd group and was characterized by acute laryngitis (38.9 % vs. 13.2 %, $\chi^2 = 4.80$; $p<0.05$) and wheezing (61.1 % vs. 18.4 %, $\chi^2 = 10.21$; $p<0.01$). The children of the 2nd group after 1 year had recurrent acute bronchitis and laryngitis, the average frequency of which was 3.6±0.2 per year.

Microbiological examination (Table II) showed that in children of the 1st and 2nd groups in comparison with the control was dominated by gram-negative and mixed microbiota.

Gram-negative microbiota was identified in 26.3 % of samples in children of 1st group and in 44.5 % of samples in children of 2nd group and was not detected in any samples in the control group.

There was no significant difference between the representatives of the gram-negative microbiota in children of the 1st and 2nd groups. *Klebsiella pneumoniae* (15.8 %) and *Escherichia coli* (10.5 %) were found among the representatives of gram-negative microbiota in children of the 1 st group (Fig 1). *Klebsiella pneumoniae* (22.2 %), *Proteus vulgaris* (11.1%) and *Proteus spp.* (5.6%) and *Escherichia coli* (5.6 %) were found in children of the 2 nd group.

The mixed microbiota was identified in 21.1 % of samples in children of 1st group and in 55.6 % of samples in children of 2nd group and was not detected in any samples in the control group.

Staphylococcus aureus was the most common among representatives of gram-positive opportunistic pathogens. It was detected in 31.6 % of samples in children of the 1st group, in 27.8 % of samples in children of the 2nd group and in 17.7 % of samples in children of the control group, but no significant difference was found.

The representative of the normal microbiota *Streptococcus viridans* was significantly less frequently identified in children of the 2nd group (11.1 %), compared with the 1st (26.3 %) and control (52.9 %) groups.

Candida albicans was significantly more often identified in children of the 1st (36.8 %) and 2nd groups (38.9 %) compared to the control group (5.6 %).

There were analyzed 178 salivary samples in children for the presence of pepsin. The test was positive in 102 (89.5 %) of 114 samples in the 1st group, in 48 (83.3 %) of 54 samples in the 2nd group and only in 10 (19.6 %) samples of 51 in the control group. The average daily level of pepsin (Table III was significantly higher in children of the 2nd group than in children of the 1st and control groups (672.0±60.6 pg/ml vs. 456.8±56.9 pg/ml and 28.5±11.6 pg/ml, $p<0.05$).

Since saliva IL-8 is a cytokine that play an important role in pathogenesis of inflammatory and autoimmune diseases, based on the positive relationship between IL-8 and neutrophils in patients suffering from pulmonary diseases, the aim of this study was also to investigate the level of saliva IL-8. The level of saliva IL-8 was significantly higher in children of the 2nd group compared to the 1st one (720.1±50.1 pg/ml vs. 331.2±22.6 pg/ml, $p<0.05$) (Figure 2).

The presented study shows the positive correlation between saliva IL-8 and pepsin level in children of 2nd group ($r=0.78$, $p<0.05$) (Figure 3). The relationship of this salivary cytokine with pepsin levels can cause inflammation and the development of lesions of mucosal lesions.

We also found an association of IL-8 levels with the frequency of acute bronchitis in children of 2nd group ($r=0.73$, $p<0.05$).

In children of the 1st group, the level of saliva IL-8 did not correlate with the pepsin level ($r=0.43$, $p>0.05$).

DISCUSSION

LPR is one of the most common and important disorders of upper airway inflammation. In contrast to GER, LPR can cause chronic laryngeal damage with as few as three reflux episodes per week. Laryngeal mucosal barriers to reflux are significantly weaker than gastroesophageal barriers. Studies implicate pepsin exposure in the damage of laryngeal tissues. Pepsin can accumulate in laryngeal tissue after exposure via receptor-mediated endocytosis. It is postulated that pepsin is activated in the acidic intravesicular environment after endocytosis. In addition, pepsin causes intracellular damage to mitochondria in cultured hypopharyngeal and nasal epithelial cells and changes the expression of genes expressed in stress and toxicity that may correlate to the mechanism of nonacidic reflux injury in LPR [12]. Moreover, the epithelial cells of the throat are susceptible to pepsin even in a non-acidic environment because pepsin stimulates the expression of many proinflammatory cytokines [4]. Cytokines regulate many aspects of the immune response, therefore, along with other factors, they will be useful tools for diagnosing and monitoring the oral cavity, and saliva can be used as diagnostic

material to measure biomarkers released during disease onset and progression [11].

The results of our study established a much higher mean level of saliva pepsin of the patients with LPR than in the GER and control group. As pepsin is excreted only in the gastrointestinal tract below the level of the pharynx, all these results indicate greater retrograde movement of the gastric contents to the level of the upper aerodigestive tract in patients with LPR than in the control group. We also found the positive correlation between pepsin level, saliva IL-8 and frequency of respiratory pathology in children with LPR. IL-8 plays an important role in immunity of the oral cavity. IL-8 is a suspected mediator of reflux-induced esophagitis and exposure of hypopharyngeal cells to pepsin at pH 7 has been shown to induce production of the neutrophil chemoattractant IL-8 in vitro, suggesting a potential role for IL-8 in reflux-mediated inflammation. IL-8 is also elevated in airway secretions in acute severe asthma and observations of negative correlation between sputum IL-8 and forced expiratory volume (FEV1) have been reported in patients with severe asthma [13]. McNally P. et all found an association of high pepsin level and higher IL-8 concentrations in BAL fluid in children with cystic fibrosis (CF). These data suggest that GER is common in children with CF and aspiration of gastric contents is associated with more pronounced lung inflammation [14].

There is a hypothesis that salivary enzymes, inflammatory molecules and peripheral mononuclear cells present in saliva may modify the respiratory epithelium and promote colonization by respiratory pathogens. Oral microbiome and its balance play a major role in an individual's general homeostasis. Any disruption leads to an increase in certain bacterial species, especially Gram-negative ones, associated with the massive production of pro-inflammatory cytokines, which causes or maintains chronic low-grade inflammation [15].

Our research showed significant alterations in the oral microbiome of patients with GER and LPR as compared to healthy control. We found that gram-negative microbiota such as *Klebsiella pneumoniae, Escherichia coli, Proteus vulgaris* and *Proteus spp.* and *Candida albicans* were identified in children with GER and LPR compared to the healthy control. At the same time, we found that in children with LPR, the amount of such a representative of the normal microbiome as *Streptococcus viridans* is sharply reduced. The differences in oral microbiome in patients with GER and LPR as compared to healthy control may be a result of changes in saliva physical and chemical properties and may have effects on airway conditions. Oral bacteria that colonize the oropharynx may be aspirated in the lower respiratory tract, particularly in individuals at high risk of infection such as patients with dysphagia and can lead to recurrent respiratory pathology.

CONCLUSIONS

Our study confirmed that the oral microbiome has features in children with GER and LPR. In young children with physiological GER there is a significant decrease in the normal microflora (*Streptococcus viridans*) compared to the control, as well as the appearance of colonies of gram-negative microbiota. The features of the microbiota in older children with LPR are the almost absence of normal microflora and the dominance of gram-negative opportunistic pathogens (*Klebsiella pneumoniae, Proteus vulgaris and Proteus spp.*) or mixed flora.

The study was established a much higher mean salivary pepsin level of the patients with LPR than in the GER and control group. We found the positive correlation between pepsin level, saliva IL-8 and frequency of respiratory pathology in children with LPR.

Thus, our study confirms that high salivary pepsin levels lead to changes in the oral microbiome, which is a risk factor for recurrent respiratory diseases in children with LPR. The potential identification of both specific enzymatic patterns and microbiome alterations may lead to the development of more individualized treatment plans.

REFERENCES

1. Curien-Chotard M., Jantchou P. Natural history of gastroesophageal reflux in infancy: new data from a prospective cohort. BMC Pediatr. 2020;20(1):152. doi:10.1186/s12887-020-02047-3.
2. Liu D., Qian T., Sun S. et al. Laryngopharyngeal Reflux and Inflammatory Responses in Mucosal Barrier Dysfunction of the Upper Aerodigestive Tract. J Inflamm Res. 2020;13:1291-1304. doi: 10.2147/JIR.S282809.
3. Lechien J.R., De Vos N., Everard A. et al. Laryngopharyngeal reflux: The microbiota theory. Med Hypotheses. 2021;146:110460. doi: 10.1016/j.mehy.2020.110460.
4. Górecka-Tuteja A., Jastrzębska I., Składzień J. et al. Laryngopharyngeal Reflux in Children with Chronic Otitis Media with Effusion. J Neurogastroenterol Motil. 2016;22(3):452-8. doi: 10.5056/jnm16013.
5. Bulmer D.M., Ali M.S., Brownlee I.A. et al. Laryngeal mucosa: its susceptibility to damage by acid and pepsin. Laryngoscope. 2010;120(4):777-82. doi: 10.1002/lary.20665.

6. Formánek M., Jančatová D., Komínek P. et al. Laryngopharyngeal reflux and herpes simplex virus type 2 are possible risk factors for adult-onset recurrent respiratory papillomatosis (prospective case-control study). Clin Otolaryngol. 2017;42(3):597-601. doi: 10.1111/coa.12779.
7. Dev Bardhan K., Strugala V., Dettmar P.W. Reflux Revisited: Advancing the Role of Pepsin. International Journal of Otolaryngology. 2012:13. doi:10.1155/2012/646901.
8. Ocak E., Kubat G., Yorulmaz İ. Immunoserologic pepsin detection in the saliva as a non-invasive rapid diagnostic test for laryngopharyngeal reflux. Balkan Med J. 2015;32(1):46-50. doi:10.5152/balkanmedj.2015.15824.
9. Samuels T.L., Johnston N. Pepsin as a causal agent of inflammation during nonacidic reflux. Otolaryngol Head Neck Surg. 2009;141(5):559-63. doi: 10.1016/j.otohns.2009.08.022.
10. Tan J.J., Wang L., Mo T.T. et al. Pepsin promotes IL-8 signaling-induced epithelial–mesenchymal transition in laryngeal carcinoma. Cancer Cell Int. 2019; 19: 64. doi:10.1186/s12935-019-0772-7.
11. Gornowicz A., Bielawska A., Bielawski K. et al. E. Pro-inflammatory cytokines in saliva of adolescents with dental caries disease. Ann Agric Environ Med. 2012;19(4):711-6.
12. Luk L.J., DelGaudio J.M. Chapter 3 - Laryngopharyngeal Reflux in Chronic Rhinosinusitis: Evidence-Based Practice. Evidence-Based Clinical Practice in Otolaryngology. Elsevier. 2018, 35p.
13. Marshall S., McCann A.J., Samuels T.L. et al. Detection of pepsin and IL-8 in saliva of adult asthmatic patients. J Asthma Allergy. 2019;12:155-161. doi:10.2147/JAA.S205482.
14. McNally P., Ervine E., Shields M.D. et al. High concentrations of pepsin in bronchoalveolar lavage fluid from children with cystic fibrosis are associated with high interleukin-8 concentrations. Thorax. 2011;66(2):140-3. doi: 10.1136/thx.2009.130914.
15. Gomes-Filho I.S., Passos J.S., Seixas da Cruz S. Respiratory disease and the role of oral bacteria. J Oral Microbiol. 2010. doi:10.3402/jom.v2i0.5811.

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The Authors declare no conflict of interest.

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