MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
SUMY STATE UNIVERSITY
ACADEMIC AND RESEARCH MEDICAL INSTITUTE

Eastern Ukrainian Medical Journal

2, M. Sumtsova st., Sumy 40007, Ukraine eumj.med.sumdu.edu.ua
e-mail: eumj@med.sumdu.edu.ua ISSN: 2663-5909 (print)/2664-4231 (online)

How to cite / Як цитувати статтю: Ilchenko S, Mozheiko T, Fialkovska A. The prognostic significance of salivary pepsin level in the development of recurrent respiratory pathology in infants with rumination syndrome. *East Ukr Med J*. 2023;11(4):421-429

DOI: https://doi.org/10.21272/eumj.2023;11(4):421-429

ABSTRACT

Svitlana Ilchenko
https://orcid.org/0000-0003-2181-1833
Department of Propaedeutics of Children's Diseases and Pediatrics 2, Dnipro State Medical University, Dnipro, Ukraine

Tetiana Mozheiko
Department of Propaedeutics of Children's Diseases and Pediatrics 2, Dnipro State Medical University, Dnipro, Ukraine

Anastasiya Fialkovska
https://orcid.org/0000-0001-6004-8418
Department of Propaedeutics of Children's Diseases and Pediatrics 2, Dnipro State Medical University, Dnipro, Ukraine

THE PROGNOSTIC SIGNIFICANCE OF SALIVARY PEPSIN LEVEL IN THE DEVELOPMENT OF RECURRENT RESPIRATORY PATHOLOGY IN INFANTS WITH RUMINATION SYNDROME

Introduction. Infant rumination syndrome (IRS) is most common among infants with functional disorders of the gastrointestinal tract and can lead to the development of recurrent respiratory pathology. Pepsin is believed to play a key role in the upper airway mucosa impairment.

The objective of the study was to determine the prognostic significance of salivary pepsin level in the development of recurrent respiratory pathology in infants with rumination syndrome.

Materials and Methods. We analyzed the clinical and anamnestic data of 55 infants: the main group consisted of 38 children with apparent rumination syndrome, and the comparison group included 17 healthy children with no clinical manifestations of this syndrome. Salivary pepsin and IL-8 levels were measured three times a day by the enzyme-linked immunosorbent assay (Human Pepsin Elisa Kit and Human IL-8 ELISA Kit, USA). The study results were statistically processed using the Statistica v.6.1 software package. To assess the diagnostic significance of salivary pepsin level, ROC analysis was performed to determine the optimal cut-off point and calculate the area under the ROC curve (AUC) with 95% CI and operating characteristics of the criterion (sensitivity and specificity).

Results and Discussion. Daily monitoring of salivary pepsin in infants showed that pepsin level was significantly higher in the main group vs. the comparison group, both for average daily values and intermediate indicators. No statistically significant difference was found between the three saliva samples and the daily average pepsin level in the main group of infants, which may be indicative of latent episodes of reflux during the day. A direct correlation was found

between salivary levels of IL-8 and pepsin levels in the main group of children ($r = 0.78$, $p < 0.05$). Among the potential predictors of recurrent respiratory pathology risk in infants with rumination syndrome, the following were established: fasting pepsin level > 309.27 pg/ml, pepsin level in 1 hour after feeding > 275.73 pg/ml, and pepsin level after regurgitation > 532.31 pg/ml.

Conclusions. Fasting pepsin level > 309.27 pg/ml and pepsin level in 1 hour after feeding > 275.73 pg/ml can be used in a multiple logistic regression model to predict the risk of recurrent respiratory pathology, taking into account other clinical, anamnestic, and laboratory data. A post-regurgitation pepsin level > 532.31 pg/ml is an independent predictor and can be used alone.

Keywords: regurgitation, pepsin, recurrent respiratory pathology, children.

Corresponding author: *Svitlana Ilchenko, Department of Propaedeutics of Children's Diseases and Pediatrics 2, Dnipro State Medical University, Dnipro, Ukraine*
e-mail: ilchensv@gmail.com

РЕЗЮМЕ

Світлана Ільченко
https://orcid.org/0000-0003-2181-1833
Кафедра пропедевтики дитячих хвороб та педіатрії 2, Дніпровський державний медичний університет, м. Дніпро, Україна

Тетяна Можейко
Кафедра пропедевтики дитячих хвороб та педіатрії 2, Дніпровський державний медичний університет, м. Дніпро, Україна

Анастасія Фіалковська
https://orcid.org/0000-0001-6004-8418
Кафедра пропедевтики дитячих хвороб та педіатрії 2, Дніпровський державний медичний університет, м. Дніпро, Україна

ПРОГНОСТИЧНА ЗНАЧУЩІСТЬ РІВНЯ ПЕПСИНУ У СЕКРЕТІ РОТОВОЇ ПОРОЖНИНИ В РОЗВИТКУ РЕКУРЕНТНОЇ РЕСПІРАТОРНОЇ ПАТОЛОГІЇ У ДІТЕЙ РАННЬОГО ВІКУ З СИНДРОМ РЕГУРГІТАЦІЇ

Вступ. Синдром малюкової регургітації (СМР) найчастіше зустрічається серед дітей раннього віку з функціональними порушеннями шлунково-кишкового тракту та може призвести до розвитку рекурентної патології дихальних шляхів. Вважається, що ключову роль у пошкодженні слизової оболонки дихальних шляхів відіграє пепсин.

Метою дослідження було визначення прогностичної значущості рівня пепсину у секреті ротової порожнини в розвитку рекурентної респіраторної патології у дітей раннього віку із синдромом регургітації.

Матеріали та методи. Проведено аналіз клініко-анамнестичних даних 55 дітей раннього віку, серед яких основну групу становили 38 дітей із видимим синдромом регургітації, а групу порівняння – 17 здорових дітей без клінічних проявів цього синдрому. Рівень пепсину та інтерлейкіну-8 (ІЛ-8) визначено в секреті ротової порожнини тричі на добу методом імуноферментного аналізу («Human Pepsin Elisa Kit» та «Human IL-8 ELISA Kit», США). Статистичну обробку результатів дослідження проведено із застосуванням пакету програм «Statistica v.6.1». Для оцінки діагностичної значущості рівня пепсину у секреті ротової порожнини проводився ROC-аналіз з визначенням оптимальної точки відсікання (cut off), розрахунком площі під ROC-кривою (AUC) з 95 % ДІ та операційних характеристик критерію (чутливість і специфічність).

Результати та їх обговорення. Результати добового моніторингу пепсину в слині дітей раннього віку показали, що його рівень був достовірно вищим у дітей з регургітацією, ніж у групі порівняння, як за середньодобовими значеннями, так і за проміжними показниками. Не виявлено вірогідних відмінностей між

трьома зразками слини та середньодобовим показником пепсину в дітей основної групи, що може свідчити про приховані епізоди рефлюксу. Виявлено прямий кореляційний зв'язок між рівнем IL-8 слини та рівнем пепсину у дітей з основної групи ($r = 0,78$, $p < 0,05$). Серед потенційних предикторів ризику розвитку рекурентної респіраторної патології у дітей із синдромом регургітації були: рівень пепсину натще > 309,27 пг/мл, рівень пепсину за годину після їжі > 275,73 пг/мл та рівень пепсину після зригування > 532,31 пг/мл.

Висновки. Рівень пепсину натще > 309,27 пг/мл та рівень пепсину за годину після їжі > 275,73 пг/мл можуть бути використаними в множинній логістичній регресійній моделі для прогнозування ризику розвитку рекурентної респіраторної патології з урахуванням інших клініко-анамнестичних та лабораторних даних. Рівень пепсину після зригування > 532,31 пг/мл є незалежним предиктором і може використовуватись окремо.

Ключові слова: регургітація, пепсин, рекурентна респіраторна патологія, діти.

Автор, відповідальний за листування: *Світлана Ільченко, кафедра пропедевтики дитячих хвороб та педіатрії 2, Дніпровський державний медичний університет, м. Дніпро, Україна*
e-mail: ilchensv@gmail.com

Abbreviations
IL-8 – interleukin-8
OR – odds ratio
95% CI – 95% confidence interval
IRS – infant rumination syndrome

INTRODUCTION / ВСТУП

Infant rumination syndrome (IRS) is most common among infants with functional disorders of the gastrointestinal tract [1, 2]. According to literature sources, the prevalence of this disorder ranges from 26 to 85% [3, 4] and has no association with gender, ethnicity, and country of residence [5]. Undoubtedly, the rumination syndrome of a healthy full-term newborn reflects the anatomical and physiological features of the digestive tract development and, as a rule, disappears in 90% of children by the age of 12 months. This syndrome is most frequent at the age of 2 to 4 months (41–67% of cases), when the child begins to move actively and turn over [6]. Although in most cases functional disorders of the gastrointestinal tract resolve without intervention, they are still early-age traumatic conditions that can cause various diseases later. As is known, IRS can lead to such complications as esophagitis, acute and recurrent respiratory pathology, physical development delay, and even unexplained death in childhood [7].

IRS is believed to lead to recurrent respiratory pathology through three possible mechanisms. The most important mechanism is the direct damaging effect of gastric contents on the respiratory mucosa, which causes its swelling, hypersecretion of mucus, mucociliary dyskinesia, and stimulates the release of inflammatory mediators. Another mechanism is stimulation of the vagus nerve response, supported by the excessive vagal reactivity observed in children with reflux compared to healthy children. The third hypothesis states the association between regurgitation and changes in the oral microbiome [8, 9]. Some studies have demonstrated that the acid itself does not damage the mucosa. Pepsin is claimed to play a key role in upper airway mucosa impairment, which can explain the mechanism of laryngeal mucosa damage in non-acidic reflux [10]. Pepsin is considered the most aggressive protease in the gastroduodenal refluxate. During regurgitation, pepsin can reach extraesophageal parts, where it adheres to the epithelium [11]. Pepsin can be found in many different tissue samples, such as laryngeal mucosa, paranasal sinuses, saliva, middle ear fluid, and bronchoalveolar fluid [12]. Pepsin remains active at pH 6.5 and up. It is then inactivated but still stable and can be reactivated when the pH changes. Recent studies have shown that pepsin can also be

reactivated in an acidic intracellular environment after receptor-mediated uptake of pepsin by laryngeal epithelial cells, even when the pH reaches 7.4. In addition, laryngeal epithelial cells are sensitive to pepsin even in a non-acidic environment, as pepsin stimulates the expression of many pro-inflammatory cytokines and receptors, such as IL-6, IL-8, TNF-α, etc. [9, 13].

To date, there is insufficient data on salivary pepsin levels in infants and their role in the development of recurrent respiratory pathology.

The objective of the study was to determine the prognostic significance of salivary pepsin level in the development of recurrent respiratory pathology in infants with rumination syndrome.

Materials and Methods

We analyzed the clinical and anamnestic data and immunoenzymatic test results of 55 infants: the main group consisted of 38 children (average age – 6.8 ± 0.9 months) with apparent rumination syndrome, and the comparison group included 17 healthy children (average age – 5.7 ± 0.3 months) with no clinical manifestations of this syndrome. The subgroup of children with recurrent respiratory symptoms (n = 19) included those with recurrent laryngitis and bronchitis during the year of observation. Salivary pepsin level was measured three times a day: in children of the main group (n=38) — in fasting state, immediately after regurgitation, and 1 hour after feeding; in children of the comparison group (healthy children, n = 17) — in fasting state, half an hour before feeding and 1 hour after feeding. In the obtained samples, each of 1 ml, pepsin level was measured by the enzyme-linked immunosorbent assay using the Human Pepsin Elisa Kit (Elabscience, USA). Along with pepsin measurements, saliva pH was measured using pH test strips with a range of 0 to 14 and 0.5 accuracy. Saliva IL-8 level was measured using a solid-phase enzyme-linked immunosorbent assay kit (Human IL-8 ELISA Kit, Elabscience, USA).

Recurrent respiratory pathology risk was predicted using univariate logistic regression to calculate the odds ratio (OR) and 95% confidence interval (95% CI). The prognostic ability of pepsin as a predictor of recurrent respiratory pathology risk was assessed. To assess the diagnostic significance of salivary pepsin level, ROC analysis (Receiver Operating Characteristic) was performed to determine the optimal cut-off point and calculate the area under the ROC curve (AUC) with 95% CI and operating characteristics of the criterion (sensitivity and specificity). A comparison of ROC curves was carried out according to the method of DeLonghi et al. [14]. The predictor values with the highest Youden index (sum of specificity and sensitivity) were selected as cut-off points (the predictor value used to classify the maximum percentage of observations correctly). The results were considered statistically significant at $p < 0.05$, and a trend was determined at $p < 0.10$.

The study was approved by the local Committee on Biomedical Ethics in accordance with the fundamental moral and ethical principles and the requirements to consider the rights, interests, and personal dignity of the study participants, which are provided by the following regulatory documents: The Declaration of Helsinki, the Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine, Good Clinical Practice (GCP), the UNESCO Universal Declaration on Bioethics and Human Rights, the Constitution of Ukraine (Articles 3, 21, 24, 28, 32), Fundamentals of the Legislation of Ukraine on Health Care (Articles 43.1, 44.1).

Results

As a part of the daily monitoring of salivary pepsin levels in children, we analyzed 165 biomaterial samples. The test was positive in 102 (89.5%) of 114 samples in the main group and only in 10 (19.6%) of 51 samples in the comparison group. Pepsin level was significantly higher ($p < 0.001$) in the main group children vs. the comparison group children, both for average daily values and intermediate indicators (Table 1).

The maximum pepsin level in the children of the main group was observed immediately after regurgitation and equaled 446.8 (267.9–534.7) pg/ml on average. No statistically significant difference was found between the three saliva samples and the daily average pepsin level in the main group, which may be indicative of latent episodes of reflux during the day. We found out that the average daily values of saliva pH were 8.1±0.4 in the children of the main group. No significant correlations were established between pepsin level and saliva pH ($p > 0.05$).

In the children with recurrent respiratory pathology, the level of IL-8 in saliva was significantly higher than in the comparison group (720.1 ± 50.1 pg/ml vs. 331.2 ± 22.6 pg/ml, $p < 0, 05$). A direct correlation was found between salivary levels of IL-8 and pepsin levels in the main group of children ($r = 0.78$, $p < 0.05$).

Table 1 – Average values of pepsin levels in children of the main group vs. comparison group

Salivary pepsin level, pg/mL		Main group (n = 38)	Comparison group (n = 17)	*p*
Portion I	Min-Max	139.4–968.1	0.0–359.4	< 0.001
	Me	393.5	0.0	
	25–75%	257.7–615.3	0.0–0.0	
Portion II	Min-Max	169.9–2542.8	0.0–342.4	< 0.001
	Me	446.8	0.0	
	25–75%	267.9–534.7	0.0–0.0	
Portion III	Min-Max	172.9–807.7	0.0–356.4	< 0.001
	Me	378.9	0.0	
	25–75%	247.1–581.2	0.0–0.0	
Average daily level	Min-Max	139.4–1183.8	0.0–141.7	< 0.001
	Me	398.1	0.0	
	25–75%	255.3–608.3	0.0–28.7	

An univariate logistic regression model was constructed to achieve the study's goal. In this model, we tested the prognostic ability of salivary pepsin level to predict the development of recurrent respiratory pathology in infants with rumination syndrome. Among the listed indicators, the following parameters had statistical significance: "pepsin level after regurgitation": OR (95% CI) – 1.005 (1.0–1.009), p = 0.004; "fasting pepsin level": OR (95% CI) – 1.01 (1.004–1.02), p = 0.0001; "pepsin level in 1 hour after feeding": OR (95% CI) – 1.01 (1.004–1.02), p = 0.0001 (Fig. 1).

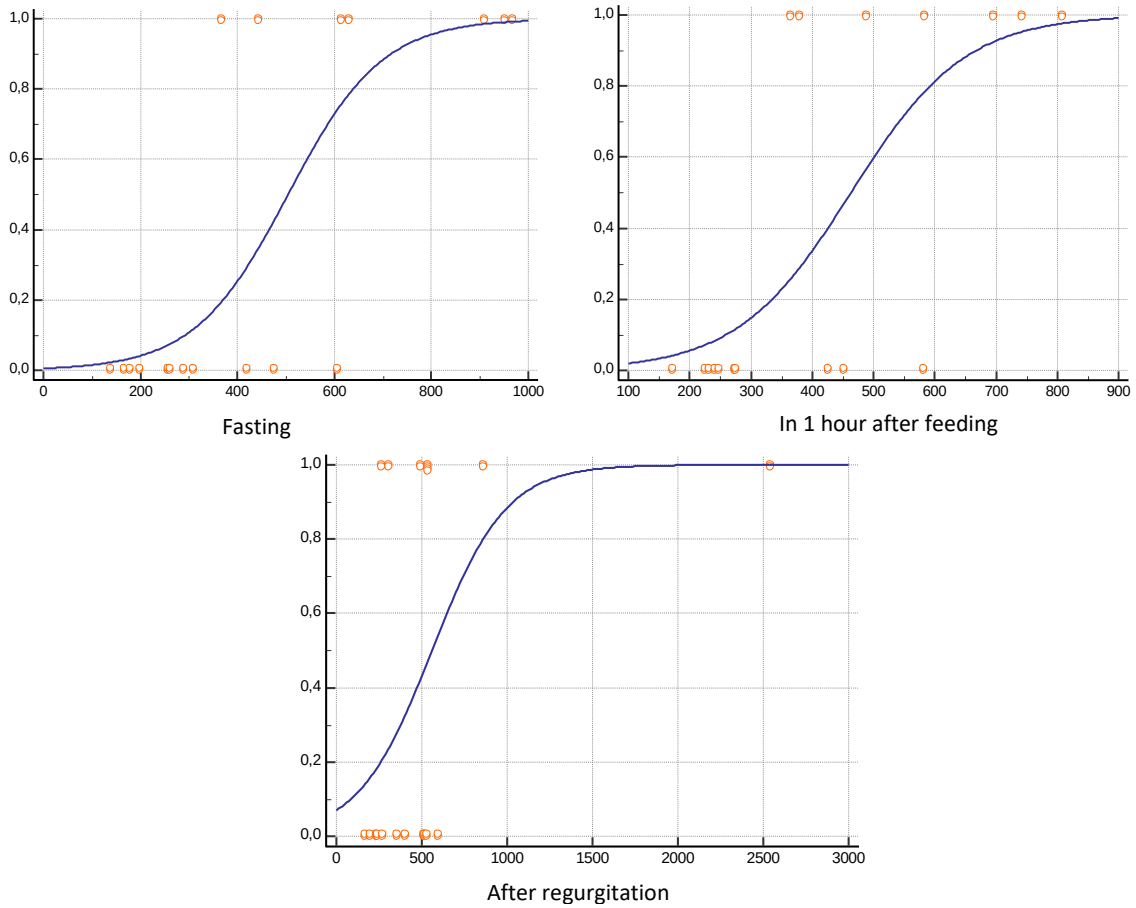

Figure 1 – Salivary pepsin level as a predictor of recurrent respiratory pathology in infants with rumination syndrome

At the next stage, optimal pepsin levels for the most accurate prediction of recurrent respiratory pathology risk were determined using ROC analysis. For fasting pepsin level indicator, the cut-off point was > 309.27 pg/ml (sensitivity 100%, specificity 72.7%, area under the curve = 0.935, p < 0.0001); for pepsin level in 1 hour after feeding indicator, the cut-off point was > 275.73 pg/ml (sensitivity 100%, specificity 70%, area under the curve = 0.9, p = 0.001); for pepsin level after regurgitation indicator, the cut-off point was > 532.31 pg/ml (sensitivity 57.1%, specificity 90.9%, area under the curve = 0.753, p = 0.003) (Fig. 2).

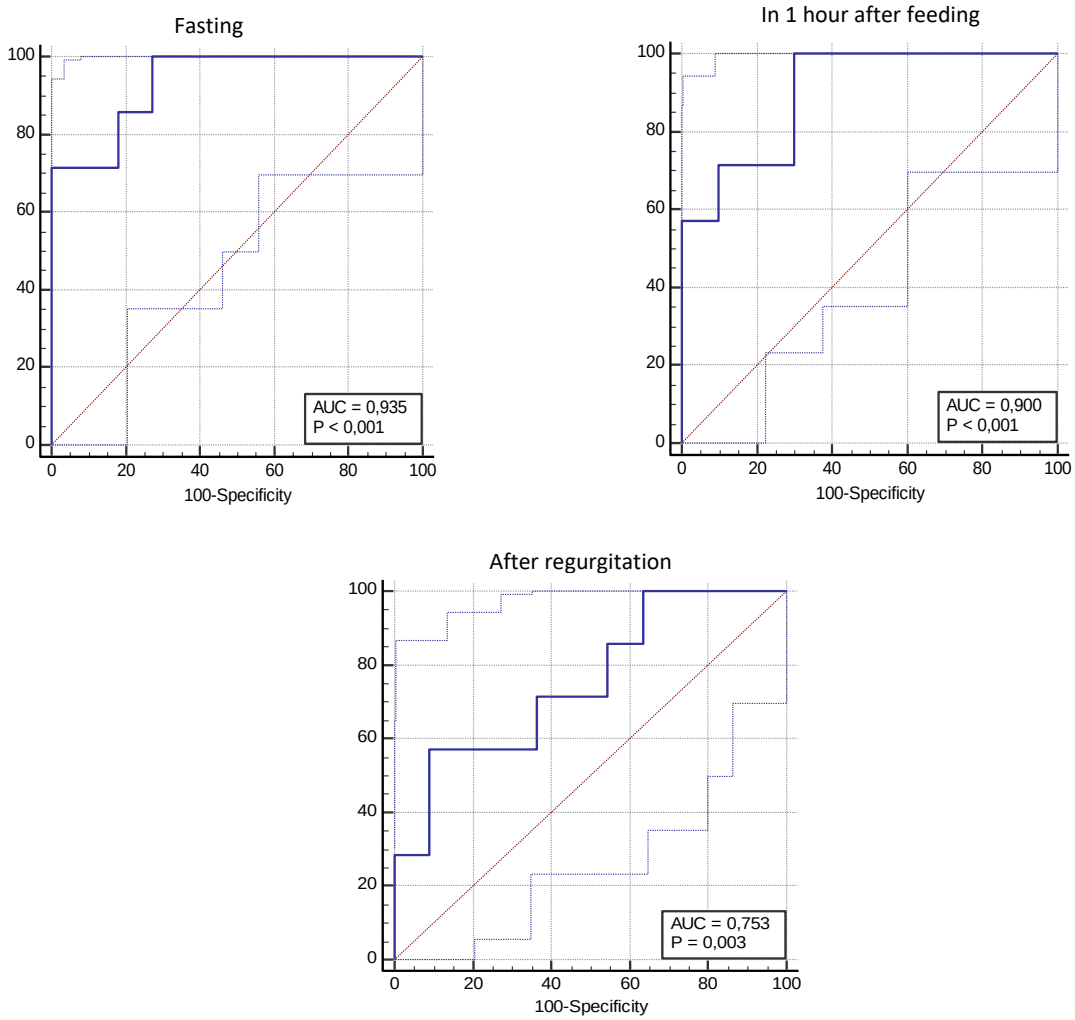

Figure 2 – ROC-curves of prognostic significance of pepsin levels a) in fasting state; b) in 1 hour after feeding; c) after regurgitation to predict recurrent respiratory pathology risk in infants with rumination syndrome

Further, a comparison of ROC curves was conducted to assess the prognostic significance of salivary pepsin levels measured at different time points (Fig. 3). Fasting pepsin level was found to have the best operating characteristics; however, this difference was not statistically significant. On the other hand, there was a trend toward a statistically significant difference between the predictive significance of fasting pepsin level and post-regurgitation pepsin level (p = 0.08).

Figure 4 presents a regression model for predicting recurrent respiratory pathology risk in infants with rumination syndrome using fasting pepsin levels. Regression equation y = -0.227+0.00135x, p < 0.001.

At the next stage, all potential predictors that showed statistical significance in an univariate logistic regression model as prognostic risk factors for recurrent respiratory pathology in infants with rumination syndrome (fasting pepsin level > 309.27 pg/ml, pepsin level in 1 hour after feeding > 275.73 pg/ml, and pepsin level after regurgitation > 532.31 pg/ml) were included in the multiple logistic regression model; while the indicators with p > 0.10 were excluded from the model in a backward stepwise way. Indicators were excluded until the value for all predictors in the model equaled p < 0.10.

The p-value of the multiple regression model remained < 0.10 after the inclusion of the post-regurgitation pepsin level > 532.31 pg/ml. AUC was 0.736 (0.557–0.871), p = 0.003. Fasting pepsin level and pepsin level in 1 hour after feeding were not included in this model; thus, according to the results of this analysis, only pepsin level after regurgitation > 532.31 pg/ml was found to be an independent predictor of recurrent respiratory pathology risk.

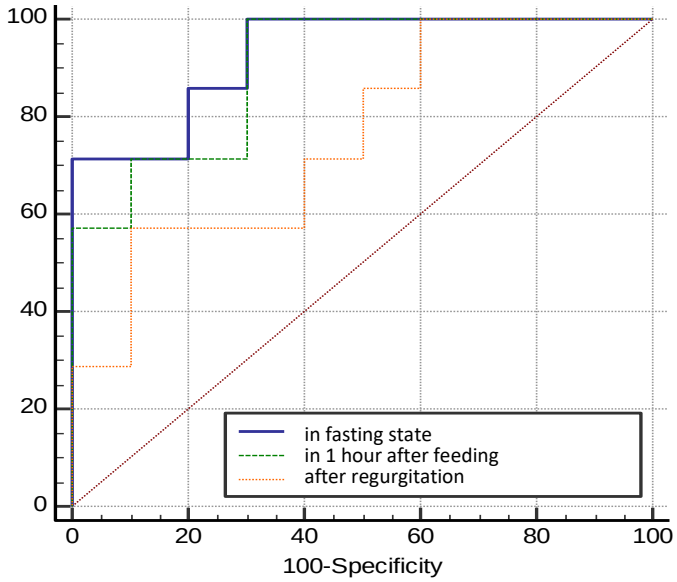

Figure 3 – Comparison of the ROC curves of prognostic significance of pepsin levels measured at different time intervals as a predictor of recurrent respiratory pathology risk

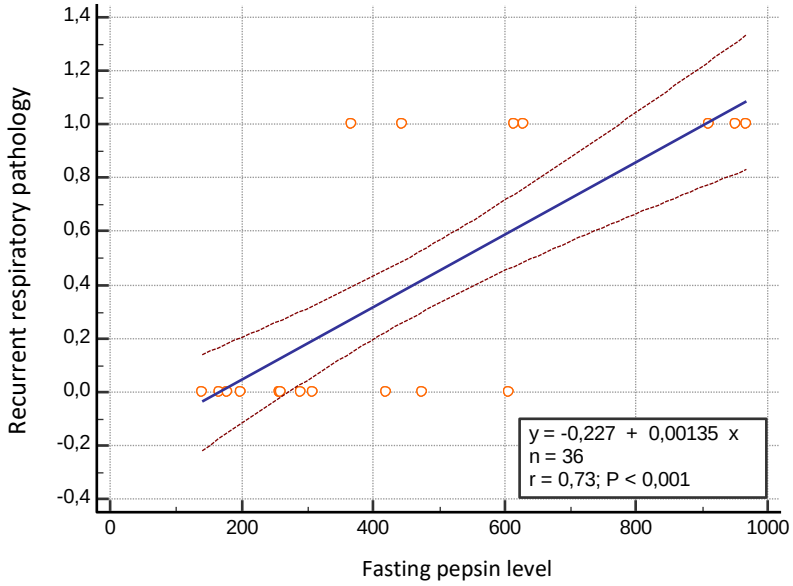

Figure 4 – Regression model for predicting recurrent respiratory pathology risk using fasting pepsin levels

Discussion

Our study has shown that pepsin levels were significantly higher in infants with regurgitation vs. comparison group, both for average daily values and intermediate indicators. The maximum pepsin level in children of the main group was observed immediately after regurgitation. No statistically significant difference was found among the three saliva samples and the average daily pepsin level in the main group of infants, which may be indicative of latent episodes of reflux during the day. In our opinion, repeated episodes of latent reflux lead to pepsin activation in the cells of the respiratory tract and, as a result, to their damage and the expression

of pro-inflammatory cytokines, particularly IL-8. Since salivary IL-8 is a cytokine that plays an important role in the pathogenesis of inflammatory and autoimmune diseases, based on the positive association between IL-8 and neutrophil levels in patients with pulmonary diseases [15, 16], we concluded that salivary level of IL-8 was significantly higher in the infants with recurrent respiratory pathology. A direct correlation was found between salivary levels of IL-8 and pepsin levels in the main group of children. Clarke D. et al. found an association between high levels of pepsin and higher IL-8 levels in bronchoalveolar lavage fluid in children with cystic fibrosis. These data suggest that gastroesophageal reflux is common in children with cystic fibrosis, and aspiration of gastric contents is associated with more pronounced lung inflammation [16].

It is hypothesized that salivary enzymes, inflammatory molecules, and peripheral mononuclear cells in saliva may modify the respiratory epithelium and promote colonization by respiratory pathogens. The oral microbiome and its balance play an important role in overall human homeostasis. Any violation leads to an increase in certain types of bacteria, especially gram-negative bacteria, associated with massive production of pro-inflammatory cytokines, thus causing or maintaining low-grade chronic inflammation [17].

Thus, measuring the salivary level of pepsin is a non-invasive method for airway reflux diagnosis, which is best among other more invasive methods, such as 24-hour pH-impedancemetry.

CONCLUSIONS / ВИСНОВКИ

1. Daily monitoring of salivary pepsin in infants showed that pepsin level was significantly higher in the main group of children vs. the comparison group of children, both for average daily values and intermediate indicators. A direct correlation was also found between salivary levels of IL-8 and pepsin levels in the main group of children ($r = 0.78$, $p < 0.05$).

2. Among the potential predictors of recurrent respiratory pathology risk in infants with rumination syndrome, the following were established: fasting pepsin level > 309.27 pg/ml, pepsin level in 1 hour after feeding > 275.73 pg/ml, and pepsin level after regurgitation > 532.31 pg/ml.

3. Fasting pepsin level > 309.27 pg/ml and pepsin level in 1 hour after feeding > 275.73 pg/ml can be used in a multiple logistic regression model to predict the risk of recurrent respiratory pathology, considering other clinical, anamnestic, and laboratory data. A post-regurgitation pepsin level > 532.31 pg/ml is an independent predictor and can be used alone.

PROSPECTS FOR FUTURE RESEARCH / ПЕРСПЕКТИВИ ПОДАЛЬШИХ ДОСЛІДЖЕНЬ

Prospects for further research consist in the analysis of the implementation of the non-invasive diagnostic method based on salivary pepsin level for a larger population and the development of preventive measures against recurrent respiratory pathology in infants with rumination syndrome..

CONFLICT OF INTEREST / КОНФЛІКТ ІНТЕРЕСІВ

The authors declare no conflict of interest.

FUNDING / ДЖЕРЕЛА ФІНАНСУВАННЯ

The work was carried out as part of the research conducted at the Department of Pediatric Diseases of Dnipro State Medical University "Bronchitis in children with comorbid diseases: clinical course and their modern etiology," state registration number 0116U004962.

AUTHOR CONTRIBUTIONS / ВКЛАД АВТОРІВ

All authors substantively contributed to the drafting of the initial and revised versions of this paper. They take full responsibility for the integrity of all aspects of the work.

REFERENCES/СПИСОК ЛІТЕРАТУРИ

1. Belousova OYu, Kazaryan LV. Functional gastrointestinal disorders in infants: to treat, observe or correct? *Child`s Health*. 2020; 15 (1):24-29. https://doi.org/10.22141/2224-0551.15.1.2020.196754

2. Rouster AS, Karpinski AC, Silver D, Monagas J, Hyman PE. Functional Gastrointestinal Disorders Dominate Pediatric Gastroenterology Outpatient Practice. *J Pediatr Gastroenterol Nutr.*

2016;62(6):847–851. https://doi.org/10.1097/MPG.0000000000001023.
3. Vandenplas Y, Rudolph CD, DiLorenzo C et al. Pediatric gastroesophageal reflux clinical practice guidelines: joint recommendations of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN). *J. Pediatr. Gastroenterol. Nutr.* 2018;49:498-547.
4. Van Tilburg M, Hyman PE, Rouster A et al. Prevalence of functional gastrointestinal disorders in infants and toddlers. *J. Pediatr.* 2015;166:684-689. http://dx.doi.org/10.1016/j.jpeds.2014.11.039.
5. Benninga MA, Nurko S, Faure C et al. Childhood Functional Gastrointestinal Disorders: Neonate/Toddler. *Gastroenterology.* 2016;150:1443-1455. https://doi.org/10.1053/j.gastro.2016.02.016.
6. Vandenplas Y, Rudolph CD, DiLorenzo C et al. Pediatric gastroesophageal reflux clinical practice guidelines: joint recommendations of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN). *J. Pediatr. Gastroenterol. Nutr.* 2018;49:498-547.
7. Ilchenko SI, Mozheiko TV, Kramarenko NM, Duplenko NV. Clinical and functional portrait of infants with regurgitation syndrome. *Child`s Health.* 2019;14(6):8-14. http://dx.doi.org/10.22141/2224-0551.14.6.2019.179241.
8. Lechien JR, De Vos N, Everard A et al. Laryngopharyngeal reflux: The microbiota theory. *Med Hypotheses.* 2021 Jan;146:110460. https://doi.org/10.1016/j.mehy.2020.110460.
9. Górecka-Tuteja A, Jastrzębska I, Składzień J et al. Laryngopharyngeal Reflux in Children with Chronic Otitis Media with Effusion. *J Neurogastroenterol Motil.* 2016 Jul 30;22(3):452-8. https://doi.org/10.5056/jnm16013.
10. Li Y, Xu G, Zhou B, et al. Effects of acids, pepsin, bile acids, and trypsin on laryngopharyngeal reflux diseases: physiopathology and therapeutic targets. *Eur Arch Otorhinolaryngol.* 2022;279(6):2743-2752. https://doi.org/10.1007/s00405-021-07201-w.
11. Niu K, Guo C, Teng S, et al. Pepsin promotes laryngopharyngeal neoplasia by modulating signaling pathways to induce cell proliferation. *PLoS One.* 2020;15(1):e0227408. https://doi.org/10.1371/journal.pone.0227408.
12. 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. https://doi.org/10.5152/balkanmedj.2015.15824 .
13. Doukas PG, Vageli DP, Sasaki CT, Judson BL. Pepsin Promotes Activation of Epidermal Growth Factor Receptor and Downstream Oncogenic Pathways, at Slightly Acidic and Neutral pH, in Exposed Hypopharyngeal Cells. *International Journal of Molecular Sciences.* 2021; 22(8):4275. https://doi.org/10.3390/ijms22084275.
14. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics.* September 1988; 44(3):837–45.
15. 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: https://doi.org/10.2147/JIR.S282809.
16. Clarke D, Gorman I, Ringholz F et al. Pulmonary aspiration in preschool children with cystic fibrosis. *Respiratory Research 19.* 2018:255. doi: https://doi.org/10.1186/s12931-018-0954-1.
17. Lechien JR, De Vos N, Everard A, Saussez S. Laryngopharyngeal reflux: The microbiota theory. *Med Hypotheses.* 2021;146:110460. https://doi.org/10.1016/j.mehy.2020.110460.

Received 18.09.2023
Accepted 28.10.2023

Одержано 18.09.2023
Затверджено до друку 28.10.2023

INFORMATION ABOUT THE AUTHORS / ВІДОМОСТІ ПРО АВТОРІВ

Ільченко Світлана Іванівна – д.мед.н., професор кафедри пропедевтики дитячих хвороб та педіатрії 2, Дніпровський державний медичний університет, 49044, м. Дніпро, вул. В. Вернадського, 9, тел.: (056) 766-48-48, e-mail: ilchensv@gmail.com, https://orcid.org/00Hi00-0003-2181-1833

Можейко Тетяна Вадимівна – очний аспірант кафедри пропедевтики дитячих хвороб та педіатрії 2, Дніпровський державний медичний університет, 49044, м. Дніпро, вул. В. Вернадського, 9, тел.: (056) 766-48-48, e-mail: joliedpua@gmail.com.

Фіалковська Анастасія Олександрівна - к.мед.н., доцент кафедри пропедевтики дитячих хвороб та педіатрії 2, Дніпровський державний медичний університет, 49044, м. Дніпро, вул. В. Вернадського, 9, тел.: (056) 766-48-48, e-mail:fialkovskaja.a@gmail.com, https://orcid.org/0000-0001-6004-8418.