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ABSTRACT

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METABOLIC FEATURES OF GASTROESOPHAGEAL REFLUX DISEASE IN ADOLESCENTS

The article presents our own research data on the study of metabolic characteristics of adolescents with gastroesophageal reflux disease (GERD), including obese patients. The objective of the study was to assess the metabolic characteristics of non-erosive and erosive clinical forms of GERD in adolescents and justify further differentiated correction of metabolic disorders.

Materials and Methods. 35 healthy adolescents (Group I – the controls) and 81 children aged 15–17 years with GERD (Group II – the main group) were examined, including 47 patients with non-erosive reflux disease (NERD) (Subgroup IIn) and 34 patients with erosive reflux esophagitis (ERE) (Subgroup IIe), including 9 obese girls. Metabolic features were studied by measuring the levels of 78 organic acids (OA) in urine using gas chromatography-mass spectrometry (GC-MS). The obtained results were statistically processed using generally accepted methods of medical statistics.

Results. Every 10th patient with GERD had lipoic acid and vitamins B1, B2, B3, and B5 deficiency, while 48.7% of adolescents with NERD and 2/3 of patients with ERE had vitamin B6 deficiency. An elevated orotate level (a biomarker of magnesium deficiency) was found in every third patient in Subgroup IIn and in 64.7% of patients in Subgroup IIe, which was also confirmed by the indicators of other metabolites (lactate, pyruvate, succinate, citrate, 2-ketoglutaric acid). Patients with GERD (29.8% and 52.9% in Subgroups IIn and IIe) significantly more often had elevated levels of hydroxyproline dipeptide (a marker of bone and connective tissue disorders), which were 2 times higher in ERE patients vs. NERD patients and 3 times higher in ERE patients vs. the controls. In 42.6% of patients with NERD and 85.3% of patients with ERE, especially in those with obesity (88.9%), metabolic markers of intestinal microbiome disorders were detected. They can support the inflammatory process in the esophageal mucosa and contribute to the disease's

progression and require appropriate correction.

Conclusion. In adolescents with non-erosive and erosive forms of GERD, especially if accompanied by obesity, a complex of metabolic disorders in carbohydrate, protein, and fat metabolism was identified, which requires further study and differentiated correction. The non-invasive GC-MS screening method used to measure urinary OA levels makes it possible to identify the main metabolic disorders in GERD in adolescents, allowing differential correction of disease therapy and control of its effectiveness and duration.

Keywords: adolescents, gastroesophageal reflux disease, obesity, microbiome, metabolic disorders.

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МЕТАБОЛІЧНІ ОСОБЛИВОСТІ ГАСТРОЕЗОФАГАЛЬНОЇ РЕФЛЮКСНОЇ ХВОРОБИ У ПІДЛІТКІВ

В статті наведені власні дані дослідження, що стосуються вивчення метаболічних особливостей підлітків з гастроезофагальною рефлюксною хворобою (ГЕРХ), включаючи пацієнтів з ожирінням.

Мета дослідження – вивчення метаболічних характеристик неерозивної та ерозивної клінічних форм ГЕРХ у підлітків для обґрунтування подальшої диференційованої корекції виявлених метаболічних розладів.

Матеріали і методи дослідження. Обстежено 35 здорових підлітків (I група контролю) та 81 дитину у віці 15-17 років з ГЕРХ (II основна група), з них 47 пацієнтів з її неерозивною формою (IIн підгрупа) та 34 – з ерозивним рефлюкс-езофагітом (ЕРЕ) (IIе підгрупа), в тому числі 9 дівчат з ожирінням. Особливості обміну речовин вивчали за рівнями 78 органічних кислот (ОК) в сечі методом газової хроматографії-масспектрометрії (ГХГ-МСМ). Статистична обробка отриманих результатів здійснювалась з використанням загально прийнятих методів медичної статистики.

Результати дослідження. У кожного 10 пацієнта з ГЕРХ виявлений дефіцит ліпоєвої кислоти та вітамінів $B_{1,2,3,5}$, а в 48,7 % підлітків з неерозивною формою захворювання та 2/3 хворих з ЕРЕ – недостатність віт. B_6. Підвищений рівень оротату (біомаркера дефіциту магнію) констатований у кожного 3 пацієнта IIн та 64,7 % хворих IIе-підгруп, що також підтверджується і показниками інших метаболітів (лактат, піруват, сукцинат, цитрат, 2-кетоглутарова кислота). У пацієнтів з ГЕРХ (29,8 % та 52,9 % в IIн та IIе-підгрупах) достовірно частіше виявлені підвищені рівні гідроксипроліну дипептиду (маркера порушень кісткової та сполучної тканини), які при ЕРЕ були вищі за показники хворих на неерозивну форму захворювання і підлітків в групі контролю відповідно в 2 рази та майже в 3 рази. У 42,6 % пацієнтів з неерозивною формою ГЕРХ та 85,3 % хворих на ЕРЕ, особливо у поєднанні з ожирінням (88,9 %), виявлені метаболічні маркери порушень кишкового мікробіому, які здатні підтримувати запальний процес в СО стравоходу та сприяти прогресуванню захворювання і потребують відповідної корекції.

Висновок. У підлітків з неерозивною та ерозивною формою ГЕРХ, особливо за наявності ожиріння, виявлено комплекс

метаболічних порушень у вуглеводному, білковому та жировому обміні, що потребує подальшого вивчення та диференційованої їх корекції. Скринінговий неінвазивний метод ГХГ-МСМ для визначення рівнів ОК в сечі дає можливість ідентифікувати при ГЕРХ у підлітків основні метаболічні порушення, що дозволить диференційовано корегувати терапію захворювання, контролювати її ефективність і тривалість.

Ключові слова: підлітки, гастроезофагальна рефлюксна хвороба, ожиріння, мікробіом, метаболічні порушення.

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INTRODUCTION

Gastroesophageal reflux disease (GERD) is one of the most common chronic recurrent diseases of the upper gastrointestinal tract (GI tract), when acidic gastric contents reflux into the esophagus, causing a number of esophageal and extraesophageal symptoms and/or complications (erosive reflux esophagitis (ERE), Barrett's esophagus, adenocarcinoma), which significantly impair the patient's quality of life and lead to a significant burden on the healthcare system of any country [1]. GERD is found in all population cohorts, including children and adolescents, who are the most vulnerable category of patients. The age of 15–17 is a critical period of the end of childhood, a phase of growing up, a transitional stage of a child's physical, neuro-vegetative, and psychological/mental development from puberty to adulthood. A variety of physical, emotional, and social changes determine the predisposition of adolescents to the development of various diseases, including GERD [2]. Kesavelu D. et al. (2025) in their study noted an increase in GERD frequency in adolescents up to 20%, which is comparable to the prevalence of the disease in adults [3]. At the same time, according to the data of a multicenter clinical study by Xie M. et al. (2024), children with a body mass index ≥30 kg/m² had GERD symptoms significantly more often vs. peers with normal weight [4]; the symptoms were detected with a frequency of 13% to 38% [5], and this percentage increased precisely at the age of 13–17 years [6].

According to traditional ideas about GERD formation, pathological changes in the esophageal mucosa (EM) are mainly caused by chemical damage from acidic gastric contents or duodenal reflux. Therefore, until recently, it was believed that as the disease progresses, lesions gradually cover the submucosal, muscular, and serous layers of the organ. However, in most patients with GERD, visible damage to the esophageal mucosa is not detected during esophagogastroduodenoscopy (EGD), which indicates the possible involvement of other pathogenic influences in this disease formation [7, 8]. The results of recent studies indicate that the interaction between the immune system, microbiome, and metabolic processes in the body plays a key role in the pathogenesis of various gastrointestinal diseases, including GERD [9]. At the same time, the diversity, stability, and response of the microbiome to physiological, pathological, and environmental changes make it (and the metabolic pathways associated with it) an important biomarker and diagnostic tool or therapeutic target for various diseases [1]. To date, microbiome composition and function, as well as the complex of metabolites associated with its disorders in patients with GERD, remain largely unknown. However, it has been proven that the microbiota of the GI plays an important role in maintaining its homeostasis by converting host nutrients into a number of vital metabolites, the spectrum of which has been studied in recent years using modern metabolomics technologies. These technologies are promising for characterizing pathophysiological processes in the human body, as they can dynamically detect the entire set of low molecular weight molecules in cells, tissues, organs, biological fluids, and feces [10, 11]. They allow for accurate assessment of the body's individual metabolic responses to pathophysiological stimuli (medicinal drugs, environmental changes, lifestyle, diseases, and other epigenetic factors) and help select the best therapeutic approach. In their review, the authors provide some evidence for the clinical application of metabolomics in heart diseases, hearing impairment, nephro-urological disorders, oncological diseases in adults and children, asphyxia, neonatal sepsis, and even certain neuropsychiatric disorders, including autism [10]. Regarding digestive diseases, research to date has focused mainly on inflammatory bowel diseases [11, 12], metabolic dysfunction-associated steatotic liver disease [13, 14] and cirrhosis

[15], obesity [16], and, to a much lesser extent, eosinophilic esophagitis [17] and GERD [1].

In modern routine clinical practice, a simple, informative, and noninvasive method of gas chromatography-mass spectrometry (GC-MS) is widely used to determine the organic acids (OA) levels in urine/blood for biomarker levels and metabolic homeostasis screening in patients with various diseases, including GERD [1]. OAs are a group of organic compounds that are intermediate products of metabolism in the body's cells. Their presence and concentration in urine/blood may be indicators of metabolic disorders or decreased activity of enzymes responsible for certain metabolic pathways. OAs have the properties of amino acids, contain carbon atoms in their molecule, and are formed as a result of various biochemical processes related to the metabolism of proteins, fats, and carbohydrates. OAs are excreted during blood filtration by the kidneys and are almost not reabsorbed in the renal tubules, so their concentration in the urine is always higher than in the blood. That is why it is more appropriate to measure OAs in urine to detect abnormal OA accumulation, which results from insufficient enzyme activity or gut microbiome disorders [18].

Since metabolic imbalance of various genesis can contribute to the development of a number of gastroenterological diseases, including GERD [19, 20], it is advisable to determine biomarkers of protein, fat, and carbohydrate metabolism disorders in urine to clarify the metabolic features of erosive and non-erosive clinical forms of GERD in adolescents.

STUDY OBJECTIVE

The objective of the study was to assess the metabolic characteristics of non-erosive and erosive clinical forms of GERD in adolescents and justify further differentiated correction of metabolic disorders.

MATERIALS AND METHODS

On the basis of the children's polyclinic and gastroenterology departments of the Children's Clinical Hospital No. 9 in Kyiv, 35 apparently healthy children aged 15 to 17 years were examined (18 boys (51.4%) and 17 girls (49.6%), who made up Group I (the controls)) and 81 adolescents of the same age who suffered from GERD (39 girls (48.1%) and 42 boys (51.9%), who were included in Group II (the main group)). We divided the patients of Group II into 2 subgroups depending on the clinical form of GERD: Subgroup IIn consisted of 47 adolescents with a non-erosive form – NERD (23 girls (48.9%), 24 boys (51.1%)), and Subgroup IIe included 34 patients with an erosive form of the disease – ERE (18 boys (52.9%) and 16 girls (47.1%), 9 of whom were obese).

The study was carried out as part of the dissertation research work “Clinical and pathogenetic substantiation of differentiated therapy of adolescents with gastroesophageal reflux disease”; the protocol was approved by the Bioethics Committee of the Shupyk National Healthcare University of Ukraine. Informed consent to participate in the study was obtained from all participants.

Inclusion criteria: age of 15 to 17 years; verified diagnosis of GERD; absence of severe allergic (bronchial asthma, atopic dermatitis, chronic urticaria, allergic rhinitis), immune (congenital and acquired immunodeficiency diseases), and autoimmune disorders (rheumatoid arthritis, systemic lupus erythematosus, Behçet's syndrome, Sjögren's syndrome, multiple sclerosis, cardiomyopathy, autoimmune myocarditis).

Exclusion criteria: child's age less than 15 years; no verified diagnosis of GERD; severe allergic (bronchial asthma, atopic dermatitis, chronic urticaria, allergic rhinitis), immune (congenital and acquired immunodeficiency diseases), and autoimmune disorders (rheumatoid arthritis, systemic lupus erythematosus, Behçet's syndrome, Sjögren's syndrome, multiple sclerosis, cardiomyopathy, autoimmune myocarditis).

Verification of GERD diagnosis was carried out in accordance with international recommendations [21], which became the basis of the relevant National Clinical Guidelines based on evidence and the Standard of Medical Care “Gastroesophageal Reflux Disease in Children” [22], and also included the assessment of complaints, duration of symptoms, physical examination, and medical history, since the reliability of symptom-based clinical diagnosis of GERD is particularly high in older children – they usually complain of heartburn and acid regurgitation, like adults [22]. According to the recommendations of this Standard, the disease can be suspected and diagnosed by a doctor of any specialty based on the detection of esophageal (abdominal pain/night pain, heartburn, belching/acid belching, dysphagia, pain in the epigastric region and chest, periodic vomiting, odynophagia (pain during the food passage through the esophagus), which occur more often with erosive-ulcerative lesions) and extraesophageal (dental, laryngopharyngeal, bronchopulmonary, cardiac) clinical manifestations of GERD or GER. Patients who had the following symptoms: hematomesis; melena; dysphagia; no improvement in regurgitation after 1 year (or late onset of regurgitation); progressive developmental delay associated with regurgitation; unexplained distress accompanied by communication difficulties; retrosternal, epigastric, and upper abdominal pain requiring constant medical attention or not amenable to medical treatment; a history of food aversion and

regurgitation; iron deficiency anemia of unknown genesis, etc., – were prescribed esophagogastroduodenoscopy (EGD) to verify the diagnosis of erosive reflux esophagitis (ERE) according to the criteria of the LA classification system (degree of esophagus mucosa damage) and the Savary-Miller International Endoscopic Classification of GERD (severity of RE) (as modified by Y. J. Tytgat et al.) [23], since the presence of ERE typical signs (erosions or ulcers at/or directly above the gastroesophageal junction) during fibroesophagogastroduodenoscopy is considered a diagnostic criterion for GERD and ERE, which has a specificity of 90%-95% [23]. In our study, we prescribed EGD to all examined patients to exclude other GI diseases that may have symptoms of heartburn and acid regurgitation (chronic gastritis, gastric and duodenal ulcer, sliding hernia of the esophageal hiatus).

The study of metabolic features in adolescents with GERD with regard to the clinical form of the disease, was carried out by measuring OA levels in the urine using the GC-MS method at the Department of Hereditary Disorder Diagnostics of the Medical Genetics Laboratory of the National Children's Specialized Hospital “OKHMATDYT” of the Ministry of Health of Ukraine (Certificate of Accreditation ISO 15189-2022 No. 30095 dated 30 December 2022).

In patients with NERD and ERE and apparently healthy adolescents, the levels of 78 chemical compounds in urine were analyzed by the following groups of biochemical processes:

- glycolysis and carbohydrate metabolism intermediates (2),
- markers of tricarboxylic acid cycle metabolism, cellular energy supply, mitochondrial dysfunction, B vitamins, coenzyme Q, and magnesium (6),
- markers of ketogenesis and fatty acid β-oxidation (Knoop-Linnen cycle) (17),
- tryptophan and lysine metabolites (5),
- markers of branched-chain amino acid metabolism (leucine, isoleucine, valine) (16),
- metabolites of aromatic amino acids (phenylalanine and tyrosine) (8),
- markers of neurotransmitter metabolism (4),
- markers of oxalate metabolism (2),
- markers of pyrimidine metabolism (2),
- markers of vitamin B12 and folic acid metabolism, and methylation disorders (5),
- markers of detoxification and endogenous intoxication (4),
- yeast and fungal metabolites (2),
- bacterial metabolites (3),
- bone and connective tissue metabolites (2).

Statistical processing of the obtained research results was done using the Statistica 7 software package (StatSoft, USA) according to generally accepted methods of medical variation statistics. The Student's t-test and the two-proportion comparison test were used when comparing mean values. Results were considered statistically significant at $p<0.05$. Differences between groups were assessed using the Mann-Whitney (U) test; differences between distributions were assessed using the Pearson $\chi 2$ test.

RESULTS

Data on the frequency of detected metabolic disorders and levels of metabolic markers in adolescents with NERD and ERE and schoolchildren of the same age in the control group are provided in Tables 1 and 2.

When analyzing the metabolic features of the examined adolescents, disorders of oxalate metabolism were detected in 9 (19.1%) patients with NERD and 11 (22.4%) patients with ERE, while in apparently healthy adolescents, no such disorders were found. These disorders were characterized by increased glycolic acid levels – 29.99 ± 0.73 μM/M creat. and 29.07 ± 0.37 μM/M creat. in Subgroups IIn and IIe, respectively, which was significantly higher than that in the control group (9.93 ± 3.71 μM/M creat.) ($p < 0.05$).

In 2/3 of patients with ERE (22 (64.7 %)), including 5 (55.6 %) of 9 schoolgirls with NBW and obesity, and 1/3 of patients with NERD (17 (36.2 %)) ($p < 0.05$) and in 2 (5.7 %) adolescents of the control group ($p < 0.001$), changes in pyrimidine metabolism were observed with high levels of orotic (pyrimidine-4-carboxylic) acid (orotate) in Subgroups IIn and IIe – 2.887 ± 0.701 μM/M creat. and 2.597 ± 0.805 μM/M creat., respectively, versus 0.754 ± 0.670 μM/M creat. in the control group ($p < 0.05$).

Disorders of aromatic amino acids (phenylalanine and tyrosine) metabolism were significantly more frequently detected in patients with ERE (10 (29.4%)) vs. patients with NERD (9 (19.1%)) ($p < 0.05$) and the controls (1 (2.9%)) ($p < 0.001$), which was accompanied by an increased urine content of 4-hydroxyphenyl-lactic acid (2.34 ± 0.92, 1.99 ± 0.82 and 0.59 ± 0.18 μM/M creat.) ($p < 0.05$), 4-hydroxyphenyl-pyruvic acid (5.176 ± 0.427, 4.994 ± 0.238 and 0.707 ± 0.442 μM/M creat.) ($p < 0.05$), 4-hydroxy-phenylacetic acid (2.401 ± 0.723, 2.097 ± 0.347 and 0.707 ± 0.442 μM/M creat.) ($p < 0.05$), as well as phenylpyruvic acid (0.96 ± 0.81 and 0.90 ± 0.52 μM/M creat.), while no such cases were found in the control group.

Table 1 – **Frequency of metabolic disorders when measuring organic acids in urine by the HCG-MSM method (n = 126)**

Metabolite name, reference values, and units	Group I (the controls) (n = 35)	Subgroup IIn (n = 47)	Subgroup IIe (n = 34)
	abs. number (%) [95% CI]	abs. number (%) [95% CI]	abs. number (%) [95% CI]
Markers of oxalate metabolism			
Glycolic acid [7.17–28.16 μM/M creat.]	0	9 (19.1%)	11 (22.4%)
Pyrimidine metabolism markers			
Orotic acid (pyrimidine-4-carboxylic acid) (orotate), [0.12–0.864 μM/M creat.]	2 (5.7%) *,**	17 (36.2%) ***	22 (64.7%)
of them in girls with NBW and obesity (n = 9)	0	0	*5 (55.6%)^^*
Markers of ketogenesis and fatty acid β-oxidation (Knoop-Linnen cycle)			
Isovalerylglycine [normally absent]	0	1 (2.1%) ***	7 (20.6%)
of them in girls with NBW and obesity (n = 9)	0	0	*7 (77.8%)^*
Markers of branched-chain amino acid metabolism (leucine, isoleucine, valine)			
Isovalerylglycine [normally absent]	0	1 (2.1%) ***	7 (20.6%)
of them in girls with NBW and obesity (n = 9)	0	0	*7 (77.8%)^*
Glycolysis and carbohydrate metabolism intermediates			
Lactic acid (lactate) [4.08–26.79 μM/M creat.]	3 (8.6%)	5 (10.6%)	4 (11.8%)
Pyruvic acid (pyruvate) [3.26–21.07 μM/M creat.]	3 (8.6%)	5 (10.6%)	4 (11.8%)
Bone and connective tissue metabolites			
Bone and connective tissue metabolites [0.0–550.0 μM/M creat.]	2 (5.7%) *,**	14 (29.8%) ***	18 (52.9%)
Markers of neurotransmitter metabolism			
5-hydroxyindoleacetic acid (serotonin) [24.0–995.0 μM/M creat.]	0	1 (2.1%)	0
Aromatic amino acid metabolites (phenylalanine and tyrosine)			
4-hydroxyphenyllactic acid (Tyr) [0.0–0.87 μM/M creat.]	1 (2.9%) *,**	9 (19.1%)	8 (23.5%)
4-hydroxyphenylacetic acid (Tyr) [0.045–1.563 μM/M creat.]	1 (2.9%) *,**	6 (12.8%) ***	8 (23.5%)
4-hydroxyphenylpyruvic acid (Tyr) [0.338–4.683 μM/M creat.]	(2.9%) *,**	9 (19.1%)	8 (23.5%)
Phenylpyruvic acid (Phe) [normally absent]	0	12 (25.5%)	9 (26.5%)
Tryptophan and lysine metabolites			
Kynurenic acid (Trp) [normally absent]	0	23 (48.9%) ***	21 (61.8%)
Markers of tricarboxylic acid cycle metabolism, cellular energy supply, mitochondrial dysfunction, B vitamins, coenzyme Q, and magnesium			
Citric acid (citrate) [46.76–360.01 μM/M creat.]	1 (2.9%) *,**	5 (10.6%)	3 (8.8%)
2-ketoglutaric acid [0.681–4.493 μM/M creat.]	1 (2.9%)	2 (4.2%)	1 (2.9%)
Succinic acid (succinate) [1.50–10.73 μM/M creat.]	2 (5.7%)	3 (6.4%)	2 (5.9%)
Bacterial metabolites (marker of intestinal microbiome disorders)			
3-hydroxyphenylpropanoic acid [normally absent]	0	20 (42.6%) ***	29 (85.3%)
of them in girls with NBW and obesity (n = 9)	0	0	*8 (88.9%)^^*
Benzoic (dracylic) acid [0.0–2.14 μM/M creat.]	0	17 (36.2%) ***	29 (85.3%)
of them in girls with NBW and obesity (n = 9)	0	0	*8 (88.9%)^*
Yeast and fungal metabolites (marker of intestinal microbiome disorders)			
Oxoglutaric acid [12.0–15.88 μM/M creat.]	0	7 (14.9%) ***	16 (47.1%)
of them in girls with NBW and obesity (n = 9)	0	0	*5 (55.6%)*

*Note: * – significant difference ($p < 0.05$) between the control group and Subgroup IIn; ** – between the control group and Subgroup IIe; *** – between Subgroup IIn and Subgroup IIe; ^ – between girls with NBW and obesity and patients without NBW and obesity; ^^ – between girls with NBW and obesity and adolescents of the control group*

Table 2 – **Level of metabolic disorder markers in examined adolescents when measuring organic acids in urine by the GC-MS method (n = 126)**

Metabolite name, reference values, and units	An indicator of metabolic processes and their activity	Group I (the controls) (n = 35)	Subgroup IIn (n = 47)	Subgroup IIe (n = 34)
		M ± m	M ± m	M ± m
Markers of ketogenesis and fatty acid β-oxidation				
Isovalerylglycine (Leu), [0.178–1.996 μM/M creat.]	A marker of mitochondrial dysfunction, intestinal microbiome disorders, and fatty acid β-oxidation in the Knoop-Linnen cycle	1.429 ± 0.710 *,**	2.729 ± 0.418	2.981 ± 0.607
Glycolysis and carbohydrate metabolism intermediates				
Lactic acid (lactate), [4.08–26.79 μM/M creat.]	Disorders of the pyruvate dehydrogenase cycle, deficiency of magnesium, lipoic acid, vitamins B1, B2, B3, B5	15.14 ± 6.19 *,**	29.94 ± 2.11	32.92 ± 1.99
Pyruvic acid (pyruvate), [3.26–21.07 μM/M creat.]	A marker of magnesium, lipoic acid, vitamins B1, B2, B3, B5 deficiencies	12.41 ± 5.88*,**	22.04 ± 0.38	24.90 ± 2.01
Markers of tricarboxylic acid cycle metabolism, cellular energy supply, mitochondrial dysfunction, B vitamins, coenzyme Q, and magnesium				
Citric acid (citrate) [46.76–360.01 μM/M creat.]	A marker of magnesium deficiency	122.18 ± 60.06 *,**	392.14 ± 20.08	407.53 ± 34.28
2-ketoglutaric acid [0.681–4.493 μM/M creat.]	A marker of magnesium, lipoic acid, vitamins B1, B2, B3, B5 deficiencies	1.992 ± 0.781 *,**	4.997 ± 0.481	5.242 ± 0.745
Succinic acid (succinate) [1.50–10.73 μM/M creat.]	A marker of magnesium and coenzyme Q10 deficiency	5.67 ± 4.31 *,**	11.61 ± 0.88	11.97 ± 1.04
Tryptophan and lysine metabolites				
Kynurenic acid [normally absent]	A marker of vitamin B6 deficiency	0	0.79 ± 0.18	0.93 ± 0.22
Markers of branched-chain amino acid metabolism (leucine, isoleucine, valine)				
Isovalerylglycine [0.178–1.996 μM/M creat.]	A marker of mitochondrial dysfunction, intestinal microbiome disorders, and fatty acid β-oxidation in the Knoop-Linnen cycle	1.429 ± 0.710 *,**	2.729 ± 0.418	2.981 ± 0.607
Aromatic amino acid metabolites (phenylalanine and tyrosine)				
4-hydroxyphenyl-lactic acid (Tyr), [0.0–0.87 μM/M creat.]	Intestinal microbiome disorders, vitamin C, and antioxidant deficiency	0.59 ± 0.18 *,**	1.99 ± 0.82	2.34 ± 0.92
4-hydroxyphenylpyruvic acid (Tyr), [0.338–4.683 μM/M creat.]	Intestinal microbiome disorders	0.954 ± 0.608 *,**	4.994 ± 0.238	5.176 ± 0.427
4-hydroxyphenylacetic acid (Tyr), [0.045–1.563 μM/M creat.]	Intestinal microbiome disorders	0.707 ± 0.442 *,**	2.097 ± 0.347	2.401 ± 0.723
Phenylpyruvic acid (Phe), [normally absent]	Intestinal microbiome disorders	0	0.90 ± 0.52	0.96 ± 0.81
Markers of oxalate metabolism				
Glycolic acid, [7.17–28.16 μM/M creat.]	Intestinal microbiome disorders	9.93 ± 3.71 *,**	29.99 ± 0.73	29.07 ± 0.37
Pyrimidine metabolism markers				
Orotic (pyrimidine-4-carboxylic) acid (orotate), [0.12–0.864 μM/M creat.]	A marker of magnesium, arginine, vitamin B6 deficiencies, intestinal microbiome disorders, and ammonia detoxification	0.754 ± 0.670 *,**	2.887 ± 0.701	2.597 ± 0.805
Yeast and fungal metabolites				
Oxoglutaric acid, [12.00–15.88 μM/M creat.]	Intestinal microbiome disorders	13.47 ± 1.49 *,**	18.02 ± 0.79	18.75 ± 0.81
Bacterial metabolites				
3-hydroxyphenylpropanoic acid, 2 [normally absent]	Intestinal microbiome disorders	0	0.39 ± 0.12	0.56 ± 0.17
Benzoic (dracylic) acid [0.0–2.14 μM/M creat.]	A marker of glycine and vitamin B5 deficiencies and intestinal microbiome disorders	1.18 ± 0.51 *,**	2.98 ± 0.31	3.02 ± 0.79
Bone and connective tissue metabolites				
Hydroxyproline dipeptide [0.0–550.0 μM/M creat.]	A marker of connective tissue degradation	417.01 ± 209.05 **	608.28 ± 51.09 ***	1241.0 ± 301.3

*Note: * – significant difference ($p < 0.05$) between the control group and Subgroup IIn; ** – between the control group and Subgroup IIe; *** – between Subgroup IIn and Subgroup IIe*

Changes in the metabolism of the tryptophan and lysine amino acids were found in 23 (48.9%) and 21 (61.8%) subjects in Subgroups IIn and IIe, respectively, vs. no such cases in the controls. These disorders were accompanied by significant levels of kynurenic acid – 0.79 ± 0.18 and 0.93 ± 0.22 μM/M creat., which is normally not detected in urine.

Metabolic disorders of bone and connective tissue were significantly more frequently detected in patients with ERE – 18 (52.9%) versus 4 (8.5%) patients with NERD ($p < 0.05$), while in the control group, it was found only in 1 (2.9%) person ($p < 0.001$). When analyzing the average urine levels of hydroxyproline dipeptide in patients of Subgroup IIn, a slight increase was observed (608.28 ± 51.09 μM/M creat.). In contrast, in ERE subjects, the level of this biomarker was 1241.0 ± 301.3 μM/M creat., which was almost 3 times higher than in apparently healthy adolescents (417.01 ± 209.05 μM/M creat.) ($p < 0.05$).

The frequency of detection of markers of the tricarboxylic acid cycle metabolism, cell energy supply processes, signs of mitochondrial dysfunction, activity of B vitamins, coenzyme Q, and magnesium did not significantly differ between the control group and Subgroups IIn and IIe (Table 1); however, the levels of marker metabolites were significantly higher in patients with GERD. Thus, the indicators of citric acid (citrate) in Subgroups IIn and IIe were 392.14 ± 20.08 μM/M creat. and 407.53 ± 34.28 μM/M creat. respectively, versus 122.18 ± 60.06 μM/M creat. in the control group ($p < 0.05$). The levels of 2-ketoglutaric acid were significantly higher in NERD – 4.997 ± 0.481 μM/M creat. and in ERE – 5.242 ± 0.745 μM/M creat. compared to the control group – 1.992 ± 0.781 μM/M creat. ($p < 0.05$). The same applied to succinic acid: its levels were 2 times higher than those of apparently healthy adolescents (5.67 ± 4.31 μM/M creat.) in Subgroups IIn and IIe – 11.61 ± 0.88 μM/M creat. and 11.97 ± 1.04 μM/M creat., respectively ($p < 0.05$).

Certain changes were also identified in lipid metabolism in patients with GERD. It is noteworthy that disorders of β-oxidation of fatty acids of the Knoop-Linnen cycle were significantly more common in patients with ERE and NBW and obesity – 7 subjects (77.8%) compared to patients of Subgroup IIn – 1 subject (2.1%) ($p < 0.05$), while in apparently healthy adolescents, such changes were not detected at all. This was accompanied by an increased level of isovalerylglycine (Leu) in both subgroups (2.729 ± 0.418 μM/M creat. and 2.981 ± 0.607 μM/M creat.) ($p > 0.05$), versus 1.429 ± 0.710 μM/M creat. in the control group ($p < 0.05$).

As for carbohydrate metabolism, the frequency of detection of disorder-related markers did not significantly differ in the controls and Subgroups IIn and IIe – 3 (8.6%), 5 (10.6%), and 4 (11.8%), respectively ($p > 0.05$). However, the levels of the biomarkers were significantly higher in non-erosive and erosive forms of GERD. Thus, the indicators of lactic acid (lactate) and pyruvic acid (pyruvate) were 29.94 ± 2.11 μM/M creat. and 32.92 ± 1.99 μM/M creat., respectively, versus 15.14 ± 6.19 μM/M creat. in the control group and 22.04 ± 0.38 μM/M creat. and 24.90 ± 2.01 μM/M creat. versus 12.41 ± 5.88 μM/M creat. in apparently healthy adolescents ($p < 0.05$).

Metabolites of yeast and fungi were detected only in patients with non-erosive and erosive forms of GERD – 7 (14.9%) and 16 (47.1%), respectively ($p < 0.05$), which was accompanied by an increase in oxoglutaric acid levels – 18.02 ± 0.79 μM/M creat. and 18.75 ± 0.81 μM/M creat. versus 13.47 ± 1.49 μM/M creat. in the control group ($p < 0.05$). The same applies to metabolites of bacterial life, which were also significantly more frequently detected in patients with ERE (29 (85.3%)), especially in the presence of NWB and obesity (8 (88.9%)), versus 20 (42.6%) and 17 (36.2%) (by levels of 3-hydroxyphenylpropanol and benzoic (dracylic) acids) ($p < 0.05$) in NERD, while there were no such disorders in the control group. Accordingly, the levels of 3-hydroxyphenylpropanoic acid, which normally should be absent, were 0.56 ± 0.17 μM/M creat. and 0.39 ± 0.12 μM/M creat. in Subgroups IIe and IIn ($p > 0.05$). The benzoic acid levels in these subgroups were 2.98 ± 0.31 μM/M creat. and 3.02 ± 0.79 μM/M creat., respectively, versus 1.18 ± 0.51 μM/M creat. in the control group ($p < 0.05$).

DISCUSSION

When analyzing the metabolic features of patients with different clinical variants of GERD and comparing them with those of apparently healthy adolescents, multicomponent disorders were found. The disorders related to protein, carbohydrate, and lipid metabolism and mitochondrial dysfunction, signs of connective tissue degradation, disorders of b-oxidation of fatty acids in the Knoop-Linnen cycle and the pyruvate dehydrogenase cycle, decreased activity of vitamins B, C, antioxidants, lipoic acid, coenzyme Q10, indicators of magnesium, glycine and arginine deficiencies, and disorders of the intestinal microbiome and ammonia detoxification processes. In adolescents with GERD, metabolic markers of intestinal microbiome disorders were identified. They can support the inflammatory process in the esophageal mucosa and contribute to disease progression, which is consistent with data from experimental models where chronic metabolic imbalance supports long-term inflammatory processes [37].

Verified disorders of oxalate metabolism in patients with non-erosive and erosive forms of GERD, while no

such disorders were observed in apparently healthy adolescents, indicate both oxalate dysmetabolism and intestinal microbiome disturbances in these patients.

In 2/3 of patients with ERE, including 55.6% of obese schoolgirls and 1/3 of patients of Subgroup IIn, changes in pyrimidine metabolism and high levels of orotic (pyrimidine-4-carboxylic) acid (orotate) were noted, which indirectly indicate intestinal microbiome disorders and ammonia detoxification, as well as arginine, magnesium, and vitamin B6 deficiencies. Our data are somewhat consistent with the results [16] regarding obesity-related metabolic disorders; however, the authors determined the metabolic profile of patients based on biomarker levels in the blood, not in the urine.

A number of modern studies have proven that vitamin B6 is a key factor in protein metabolism processes, such as transamination, deamination, decarboxylation, persulfation, racemization, elimination, substitution, and interconversion of beta groups. It participates in many stages of macronutrient metabolism, neurotransmitters and hemoglobin synthesis, carbohydrate and fatty acid metabolism, as well as in the expression of certain genes. Its active form, pyridoxal-5'-phosphate, is a coenzyme in over 140 enzymatic reactions [24]. In addition, it is a magnesium fixer and magnesium transporter [25], which ensures optimal bioavailability of this essential macronutrient into cells [26]. A number of studies have proven that magnesium is necessary for the vital activity of all cells and the functioning of more than 300 enzymes. In combination with ATP, it provides energy release through the activation of magnesium-dependent ATPases for the implementation of all energy-consuming and anabolic processes of the body, the synthesis of proteins, fatty acids, and lipids [27, 28]. One of the important aspects of the physiological action of magnesium is its direct effect on the formation of connective tissue [29] and the proven pathogenetic role of magnesium deficiency in the development of undifferentiated connective tissue dysplasia syndrome [30].

In ERE patients, metabolic disorders of bone and connective tissue were detected 5 times more often than in patients with the non-erosive form of the disease, while the levels of the hydroxyproline dipeptide biomarker in urine were almost 3 times higher than in apparently healthy adolescents. Our data are consistent with the results of a study [31] in adult patients with GERD; however, the authors did not take into account the clinical form of the disease.

Considering that the main component of connective tissue is collagen, which also accounts for up to 60% of the body's proteins, an increased level of its marker, hydroxyproline dipeptide, which plays a key role in collagen helix structure stability, reflects involvement in the formation of protein metabolism disorders in adolescents with GERD. Also, it may be one of the causes of lower esophageal sphincter dysfunction, contributing to the occurrence of gastroesophageal reflux, with the subsequent development of non-erosive or erosive clinical forms of the disease.

On the other hand, constant reflux of acidic stomach contents into the esophagus can lead to the development and maintenance of inflammation and collagen breakdown in the organ mucosa, accompanied by increased urinary levels of collagen degradation products, in particular, hydroxyproline dipeptide. It is known that the urinary excretion of bound hydroxyproline (dipeptide, tripeptide) reflects the collagen resynthesis processes, which intensify in diseases associated with collagen breakdown and subsequent compensatory hyperproduction. There are only isolated reports in the scientific literature on urinary hydroxyproline dipeptide levels in adults with GERD; however, no differentiation regarding the clinical variant of the disease was performed [31]. As for children and adolescents, there are no such studies in the literature.

Disorders of aromatic amino acids (phenylalanine and tyrosine) metabolism were significantly more frequent ($p < 0.05$) in patients with ERE compared to patients with NERD and the control group, and were accompanied by increased content of 4-hydroxyphenyl-lactic, 4-hydroxyphenyl-pyruvic, 4-hydroxyphenylacetic, and phenylpyruvic acid in the urine, while no such cases were observed in adolescents of the control group. All of the listed aromatic amino acids are indirect markers of intestinal microbiome disorders, and 4-hydroxyphenyl-lactic acid (Tyr) also indicates a deficiency of vitamin C and antioxidants.

Disorders of tryptophan and lysine metabolism were found in almost every second patient in Subgroup IIn and in 2/3 of patients in Subgroup IIe, while no such changes were detected in the controls. These disorders were accompanied by significant levels of kynurenic acid, which is a marker of vitamin B6 deficiency and should not normally be detected in urine.

Therefore, in adolescents with GERD, there are significant disorders in protein metabolism, including bone and connective tissue, which are also accompanied by a deficiency of vitamin C, antioxidants, vitamins of group B (B1, B2, B3, B5, B6), and arginine, as well as a deficiency of magnesium, an essential macroelement, which is involved in the regulation of all types of metabolism, intestinal microbiome disorders, and ammonia detoxification processes, and these disturbances are significant in ERE ($p < 0.05$).

The frequency of detection of markers of the tricarboxylic acid cycle metabolism, cell energy supply processes, signs of mitochondrial dysfunction, activity of B vitamins, coenzyme Q, and magnesium did not significantly differ between the control group and Subgroups IIn and IIe ($p > 0.05$); however, the levels of marker metabolites were significantly higher in patients with GERD, especially in those with ERE. This concerned the levels of citrate, a metabolic marker of magnesium deficiency; 2-ketoglutaric acid, a marker of magnesium deficiency; lipoic acid; vitamins B1, B2, B3, B5; succinic acid; and ubiquinone (coenzyme Q10).

As for carbohydrate metabolism disorder, the frequency of detection of its markers also did not significantly differ in the control group and Subgroups IIn and IIe ($p > 0.05$); however, the levels of its metabolites (lactate and pyruvate) were significantly higher in non-erosive and erosive forms of GERD ($p < 0.05$) which indicated disorders in the pyruvate dehydrogenase cycle, as well as a deficiency of magnesium, lipoic acid, and vitamins B1, B2, B3, B5.

Certain changes were also identified in lipid metabolism in patients with GERD. It is noteworthy that disorders of b-oxidation of fatty acids of the Knoop-Linnen cycle were significantly more common in patients with ERE (7 (20.6%)) and especially in those with obesity 7 (77.8%) compared to patients of Subgroup IIn (1 (2.1%)) ($p < 0.05$), while no such changes were detected in apparently healthy adolescents. This was accompanied by increased levels of isovalerylglycine (Leu), which is also a metabolic marker of mitochondrial dysfunction and disorders in the gut microbiome. Today, a number of modern studies have proven that excessive amounts of fatty acids produced by gut microorganisms when the microbiome is disrupted contribute to the aggravation of metabolic disorders and the progression of obesity and other diseases associated with it [32, 33].

In modern medicine, urinary OAs are often used as significant metabolic markers of gut microbiome disorders, as they are byproducts of microbial metabolism, and their high levels may indicate a type of yeast/fungal or bacterial overgrowth in the gut.

Metabolites of yeast and fungi, as an indirect marker of intestinal microbiome disorders, were detected only in patients with non-erosive and erosive forms of GERD and were accompanied by increased levels of oxoglutaric acid. As for the frequency of detection of bacterial life metabolites, they were also significantly more frequently detected in patients with ERE, especially in those with obesity (by the levels of 3-hydroxyphenylpropanol and benzoic (dracylic) acids), while no such disorders were found in the control group ($p < 0.05$). Our data are consistent with the results of a study [34] on significant disorders in the intestinal microbiome in patients with NBW and obesity and their negative impact on the subsequent course of the disease.

In recent years, numerous observational studies have revealed a link between the gut microbiota and a number of digestive diseases [35], in particular, GERD [1]. In their studies, Ye X. et al. (2023) performed genomic sequencing of feces and used liquid chromatography/mass spectrometry to study the spectrum of 288 different metabolites mainly involved in arachidonic acid, tyrosine, glutathione, and caffeine metabolism, in the blood of children with GERD aged 3 to 14 years. This study revealed a relationship between the intestinal microbiota and changes in the spectrum of metabolites (increased levels of 4-methoxycinnamaldehyde, 12-epi-leukotriene B4, propionyl-L-carnitine, prostaglandin A_1 and G2 against a background of decreased levels of 4-benzoic acid, gentisic acid, hydroquinone, tetradecanedioic acid, 2-phenylglycine, and theophylline). The authors noted that children with GERD had abnormalities in the metabolic pathways of arachidonic acid, tyrosine, sulfur, glycine, serine, threonine, glutathione, and caffeine against the background of intestinal microbiota imbalance [1], which is also consistent with the results of our study. In addition, in 2024, for the first time, a bidirectional Mendelian randomized analysis confirmed the genetic link between changes in the intestinal microbiota counts and the risk of GERD, which not only confirms the microecological therapy potential for the disease, but also lays the foundation for in-depth studies on the role of the intestinal microbiome in GERD etiology [36].

CONCLUSIONS

Thus, a comparative analysis of OA levels in urine measured by the GC-MS method in apparently healthy adolescents and age-matched schoolchildren with GERD allows us to draw the following conclusions.

1. In adolescents with non-erosive and erosive GERD, especially in those with obesity, a complex of metabolic disorders in carbohydrate (10.6%, 11.8%, and 11.8%), protein (36.2%, 64.7%, and 55.6%), and fat (2.1%, 20.6%, and 77.8%) metabolism was detected, which requires further study.

2. Every 10th patient with GERD had lipoic acid and vitamins B1, B2, B3, and B5 deficiency, while 48.7% of adolescents with NERD and 2/3 of patients with ERE had vitamin B6 deficiency.

3. An increased level of orotic (pyrimidine-4-carboxylic) acid (a biomarker of magnesium deficiency) was found in every third patient in Subgroup IIn and in 64.7% of patients in Subgroup IIe, which was also confirmed by the indicators of other metabolites (lactate, pyruvate, succinate, citrate, 2-ketoglutaric acid).

4. Patients with GERD (29.8% and 52.9% in Subgroups IIn and IIe) significantly more often had elevated levels of hydroxyproline dipeptide (a marker of bone and connective tissue disorders), which were 2 times higher in ERE patients vs. NERD patients and 3 times higher in ERE patients vs. the controls.

5. In 42.6% of patients with NERD and 85.3% of patients with ERE, especially in those with NBW and obesity (88.9%), metabolic markers of intestinal microbiome disorders were detected. They can support the inflammatory process in the esophageal mucosa and contribute to the disease's progression and require appropriate correction.

6. The non-invasive GC-MS screening method used to measure urinary OA levels makes it possible to identify the main metabolic disorders in GERD in adolescents, which will allow for differential correction of disease therapy and control of its effectiveness and duration.

AUTHOR CONTRIBUTIONS

Beketova HV + Moshchych OO + Malinovska DO:

A – research concept and design:	40% + 40% + 20%
B – collection and/or assembly of data:	10% + 60% + 30%
C – data analysis and interpretation:	40% + 40% + 20%
D – writing the article:	40% + 30% + 30%
E – critical revision of the article:	60% + 20% + 20%
F – final approval of the article:	60% + 20% + 20%

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ARTIFICIAL INTELLIGENCE DISCLOSURE

Not used.

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