



Laser Based ^{13}C -urea Breath Test in Quantitative Assessment of Bacterial Colonization, Severity of Inflammation and Atrophy in Gastric Mucosa

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Aim: to compare the results of ^{13}C -UBT with the data of morphological analysis of the gastric and duodenal mucosa obtained during gastroduodenoscopy in gastritis and peptic ulcer. To analyze the dependence of the results of diode laser spectroscopy (DLS) based ^{13}C -UBT on age, nosology, activity and severity of the inflammatory process, the degree of atrophy and metaplasia.

Materials and methods. The ^{13}C -UBT DLS was performed in 525 patients before the start of eradication therapy and in 196 patients 10–12 weeks after its completion. The breath test was carried out according to a standard protocol using the BSIA patent kit (Great Britain). During endoscopy, 134 patients underwent a biopsy from the upper third of the body of the stomach, the antrum (within 5 cm from the pylorus along the greater curvature) and the duodenal bulb with histological examination with hematoxylin-eosin staining, PAS-reaction and Giemsa. Histological assessment of the state of the gastric mucosa was carried out according to a modified Sydney system with an assessment of morphological changes according to a 4-point visual analog scale (0 to 3+).

Results. A total of 525 patients (301 men and 224 women) aged 15–80 years (median 39.8 ± 15.1 years) were examined. 239 (45.5 %) patients were diagnosed with chronic gastritis, chronic duodenitis (including erosive forms). In 286 (54.5 %) patients, peptic ulcer was diagnosed with localization in the stomach — in 42 (8 %), in the duodenum — in 238 (45.3 %), combined lesions — in 9 (1.7 %) cases. Based on the results of ^{13}C -UDT DLS, *H. pylori* infection was detected in 447 patients. *H. pylori* was not found in erosive gastritis in 15 %, erosive duodenitis in 21 % and chronic non-erosive gastritis in 23 %. In peptic ulcer of the stomach and duodenum, *H. pylori* was detected in 93 % and 97 %.

A direct linear relationship between the results of the breath test and the quantitative content of *H. pylori* on the surface of the gastric mucosa was obtained. The parameters of laser ^{13}C -UBT clearly correlate with the degree of severity of mononuclear infiltration of the mucosa (the strength of the connection is 0.78). When comparing the average values of laser ^{13}C -UBT with the total assessment of the degree of mucosal neutrophilic infiltration, no significant relationship was found. With a slight severity of mucosal atrophy, the result of laser based ^{13}C -UBT practically does not change; there is a tendency of a decrease in the breath test with an increase in atrophic changes.

Conclusion. Indicators of ^{13}C -UBT correlate with the quantitative content of *H. pylori* bacteria in the gastric mucosa, the severity of mononuclear infiltration of the gastroduodenal mucous, the severity of atrophic changes.

Keywords: laser based ^{13}C urease breath test, *H. pylori*, gastritis, peptic ulcer, atrophy, inflammation

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Лазерный ^{13}C -уреазный дыхательный тест в количественном определении *H. pylori*, оценке степени выраженности воспаления и атрофии слизистой оболочки желудка

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Цель. Провести сопоставление результатов ^{13}C -уреазного дыхательного теста (^{13}C -УДТ) с данными морфологического исследования слизистой оболочки желудка и двенадцатиперстной кишки, полученными при проведении эзофагогастродуоденоскопии (ЭГДС) при гастрите и язвенной болезни, проанализировать зависимость результатов лазерного ^{13}C -УДТ от возраста, нозологической формы, активности и выраженности воспалительного процесса в слизистой оболочке желудка и ДПК, степени ее атрофии и метаплазии.

Материалы и методы. Лазерный ^{13}C -УБТ выполнен у 525 больных до начала эрадикационной терапии и у 196 больных через 10–12 недель после ее окончания. Дыхательный тест проводили по стандартному протоколу с использованием набора BSIA (Великобритания). При ЭГДС 134 больным выполнена биопсия слизистой оболочки из тела желудка, антрального отдела (в пределах 5 см от привратника по большой кривизне) и луковицы ДПК с гистологическим исследованием с окраской гематоксилином и эозином, ШИК-реакцией и по Гимзе. Гистологическая оценка состояния слизистой оболочки желудка проводилась по модифицированной Сиднейской системе с оценкой морфологических изменений по 4-балльной визуально-аналоговой шкале (от 0 до 3+).

Результаты. Всего обследовано 525 больных (301 мужчина и 224 женщины) в возрасте 15–80 лет (медиана $39,8 \pm 15,1$ года). При этом у 239 (45,5 %) пациентов был установлен диагноз хронического гастрита, хронического дуоденита (включая эрозивные формы). У 286 (54,5 %) пациентов диагностирована язвенная болезнь: с локализацией в желудке — у 42 (8 %), в ДПК — у 238 (45,3 %), сочетанное поражение — в 9 (1,7 %) случаях. По результатам ^{13}C -УДТ ДЛС инфекция *H. pylori* была обнаружена у 447 пациентов. *H. pylori* при эрозивном гастрите не был обнаружен в 15 %, эрозивном дуодените в 21 % и хроническом неэрозивном гастрите в 23 %. При язвенной болезни желудка и двенадцатиперстной кишки *H. pylori* выявлен в 93 и 97 % соответственно. Получена прямая линейная связь между результатами дыхательного теста и количественным содержанием *H. pylori* в слизистой оболочке желудка. Показатели лазерного ^{13}C -УДТ коррелируют с выраженностью мононуклеарной инфильтрации слизистой и достоверно не связаны со степенью ее нейтрофильной инфильтрации. При небольшой степени атрофии слизистой оболочки результат лазерного ^{13}C -УДТ практически не меняется; при нарастании атрофических изменений наблюдается тенденция к снижению показателей дыхательного теста.

Выводы. Показатели ^{13}C -УДТ коррелируют с количественным содержанием бактерий *H. pylori* в слизистой оболочке желудка, степенью выраженности мононуклеарной инфильтрации слизистой оболочки гастродуоденальной области, выраженностью ее атрофических изменений.

Ключевые слова: лазерный ^{13}C -уреазный дыхательный тест, *H. pylori*, гастрит, язвенная болезнь, атрофия, воспаление

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Introduction

The urea breath test (UBT) based on the use of urea labeled with a stable carbon isotope ^{13}C (^{13}C -UBT) is currently recognized as one of the most effective method for non-invasive diagnosis of *Helicobacter pylori* (*H. pylori*) infection [1–3]. There are various methods for determining the isotopic ratio $^{13}\text{CO}_2/^{12}\text{CO}_2$ in exhaled air. One of them is based on the use of diode laser spectroscopy (DLS) technique [4–9]. This approach was used in our clinical studies aimed at studying *H. pylori*-associated diseases. High sensitivity, specificity and accuracy of this method were demonstrated both in the diagnosis of *H. pylori* infection (94.1 %, 100 %, 95.1 %, respectively) and in the control of the effectiveness of eradication therapy (91.7 %, 100 %, 98.1 %, respectively) in our previous works based on a comparison with the data of microbiological, histological and molecular genetic studies. The DLS ^{13}C -UBT is used in Russia as the most accurate among non-invasive methods for diagnosing this infection [6, 7, 10].

In the last 20 years, a number of studies have been conducted that assessed the relationship of urea

breath test indicators with endoscopic characteristics such as a quantitative content of *H. pylori* on the gastric mucosa and histological parameters of inflammation. Establishing such a relationship allows to assess the degree of changes in the mucosa without resorting to endoscopy with a biopsy without special need. In a number of works, a dependence with individual parameters has been established. However, the examined groups of patients was relatively small and mainly related to changes in the antrum of the stomach [11–13].

The aim of this study was to explore the possible relationship between of the ^{13}C -UBT DLS data and the indicators of biopsy analysis such as the degree of *H. pylori* mucosa contamination of stomach body, antrum, duodenal bulb, the severity and activity of inflammation in these sections, the degree of atrophic changes and metaplasia of the mucosa.

Aim of the study

To compare the results of ^{13}C -UBT with the data of morphological analysis of the gastric and duodenal mucosa obtained during gastroduodenoscopy in

gastritis and peptic ulcer. To analyze the dependence of the results of laser based ^{13}C -UBT on age, nosology, activity and severity of the inflammatory process, the degree of atrophy and metaplasia.

Materials and methods

The study was built according to the type of a multi-sample cross-sectional assessment with dynamics and was conducted on the basis of the Department of Propaedeutics of Internal Diseases, Gastroenterology and Hepatology of the Sechenov University (Moscow) in cooperation with the A.M. Prokhorov General Physics Institute of the Russian Academy of Science. The study included residents of the central region of Russia aged 15 to 80 years who applied to the clinic on their own about dyspeptic symptoms and who had clinical and endoscopic signs of chronic gastritis, duodenitis (including erosive forms), duodenal ulcer and stomach ulcers. Exclusion criteria were: the presence of pyloric stenosis and other diseases with impaired evacuation from the stomach; conditions occurring with gross violations of gastric secretion (achlorhydria); previous intake of drugs with anti-helicobacter activity (bismuth drugs, proton pump inhibitors and antibiotics) and pronounced ulcerogenic properties (corticosteroids, non-steroidal anti-inflammatory drugs, aspirin); severe violations of the liver, kidneys, as well as any other diseases in the stage of decompensation; allergies to bismuth drugs, proton pump inhibitors and antibiotics; mental disorders and the inability of the patient to follow the doctor's recommendations.

All patients underwent a general clinical examination and endoscopic examination of the upper

gastrointestinal tract when included in the study. During endoscopy, 134 patients underwent a biopsy from the upper third of the body of the stomach, the antrum (within 5 cm from the pylorus along the greater curvature) and the duodenal bulb with histological examination with hematoxylin-eosin staining, PAS-reaction and Giemsa. Histological assessment of the state of the gastric mucosa was carried out according to a modified Sydney system with an assessment of morphological changes according to a 4-point visual analog scale (from 0 to 3+) [14]. In preparations taken from the duodenum, the above signs of inflammation, the degree of mucosal atrophy, and the presence of gastric metaplasia were also evaluated.

H. pylori mucosal contamination was graded as mild (<20 bacteria/field), moderate (20–50 bacteria/field), or severe (>50 bacteria/field). The ^{13}C -UBT DLS was performed in 525 patients before the start of eradication therapy and in 196 patients 10–12 weeks after its completion. The breath test was carried out according to a standard protocol using the BSIA patent kit (Great Britain). The protocol involves taking a test breakfast (200 ml of orange juice and 2.4 g of citric acid), taking a primary sample, taking 100 mg of ^{13}C -urea diluted in 25 ml of water 10 minutes after the test breakfast, keeping the patient in a horizontal position in for 3 minutes with turns, taking a control sample 30 minutes after the test breakfast. The exhaled air was collected in sealed plastic containers with a capacity of 200 ml, the duration of the exhalation was several seconds. On the same day, an isotopic analysis of the exhaled air composition was carried out in the laboratory of laser diagnostics of the Institute of General Physics using an isotope analyzer based on tunable

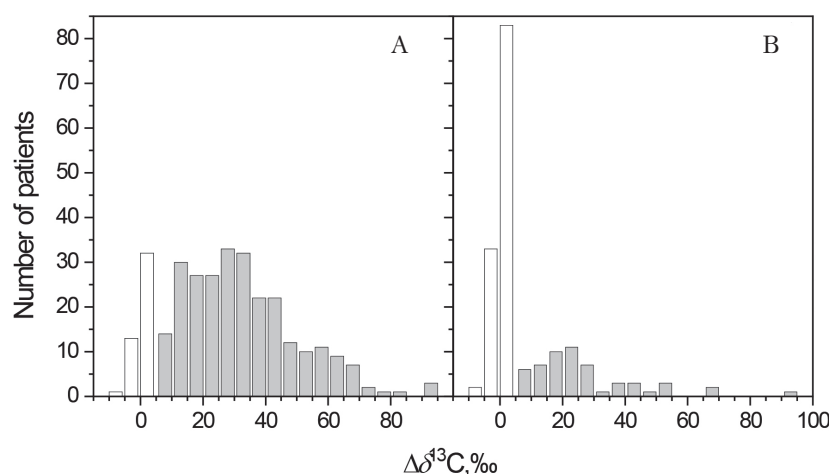


Fig. 1. Distribution diagrams for the results of the ^{13}C -urea breath test obtained before and after the eradication therapy: *A* — before treatment, *B* — after treatment; white columns — negative results below 5 ‰, gray columns are positive results

diode lasers. For each patient, the isotopic ratio $^{13}\text{CO}_2/^{12}\text{CO}_2$ was measured in the basal and control samples of exhaled air and the difference between them $\Delta\delta^{13}\text{C}$ was calculated. *H. pylori* infection was ruled out when $\Delta\delta^{13}\text{C} < 3.5\text{‰}$. The $\Delta\delta^{13}\text{C}$ values in the range of 3.5–5‰ were interpreted as falling into the “gray zone”, while the presence or absence of infection was confirmed by other additional methods. The results $\Delta\delta^{13}\text{C} > 5\text{‰}$ indicated *H. pylori* infection.

Statistical analysis. For data processing, the “Statistica” (StatSof) software package was used. Methods of nonparametric statistics were applied. Differences were considered significant at $p < 0.05$.

Research results

A total of 525 patients (301 men and 224 women) aged 15–80 years (median 39.8 ± 15.1 years) were examined. 239 (45.5 %) patients were diagnosed with chronic gastritis, chronic duodenitis (including erosive forms). In 286 (54.5 %) patients, peptic ulcer was diagnosed with localization in the stomach – in 42 (8 %), in the duodenum – in 238 (45.3 %), combined lesions – in 9 (1.7 %) cases. 525 patients initially underwent ^{13}C -UBT DLS. According to its results, infection with *H. pylori* was detected in 447 patients (Figure 1a). In 257 cases, ^{13}C -UBT DLS was repeated – 10–12 weeks after eradication anti-*Helicobacter* therapy in accordance with the recommendations of the Maastricht Consensus (Figure 1b). It follows from the figure that for the majority of patients (~80 %), the therapy was successful – *H. pylori* infection was eliminated. However, for the rest, the values of $\Delta\delta^{13}\text{C}$ exceeded 5‰ and ranged from 9 to 95‰, which corresponds to a positive *H. pylori* status.

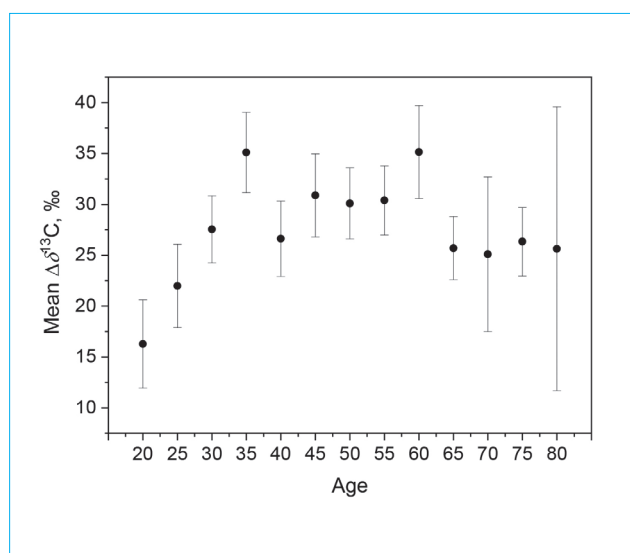


Fig. 2. Dependence of the results of ^{13}C -urea breath test on age

Distribution of the ^{13}C -UBT results by age

We analyzed the dependence of ^{13}C -UBT on age for *H. pylori*-infected patients, Figure 2. The average infection rate gradually increases at the age of 15 to 35 years. In age groups older than 60 years, there is a trend towards a decrease in the average values of $\Delta\delta^{13}\text{C}$, which is not statistically significant enough due to the small amount of data ($p > 0.05$). This indirectly confirms the participation of *H. pylori* in the gradual development of gastroduodenal pathology. The development of atrophic changes in the gastroduodenal mucosa with age leading to a deterioration in the conditions for colonization of the mucosa by *H. pylori* bacteria may explain the decrease in infection in older age groups.

Indicators of ^{13}C -urea breath test in various nosological forms

The material collected in this study allows us to identify the features of the colonization of the mucous membrane of the gastroduodenal zone in various *H. pylori*-associated diseases of stomach and duodenum. For these purposes the average values of the primary ^{13}C -UBT were obtained for various nosological groups of the examined patients (Fig. 3). As can be seen in the figure when all patients with dyspeptic complaints who underwent primary ^{13}C -UBT were included in the treatment (light columns in Fig. 3a), there is a noticeable difference in the average values for different nosological forms, for example, more than 20 % for duodenal ulcer and chronic gastritis.

This difference in the obtained average values of $\Delta\delta^{13}\text{C}$ in these groups can be explained either by the dependence of the type of disease on the degree of colonization of the gastroduodenal mucosa (which seems unlikely), or by the difference in the ratio in nosological groups between the number of infected and uninfected patients. When only positive results of ^{13}C -UBT are included in the processing, i.e. exceeding 5‰ (dark columns in Fig. 3a), the difference between the groups is significantly reduced and becomes unreliable ($p > 0.05$). This confirms the validity of the second hypothesis and also indicates a fairly good statistical sample of the results included in the processing. An analysis of the relative number of infected and uninfected patients in nosological groups showed, Figure 3b, that, indeed, each nosological form is characterized by a certain frequency of diseases not associated with *H. pylori* infection. As follows from the figure, most often the disease was not associated with *H. pylori* in erosive gastritis (~15 %), erosive duodenitis (~21 %) and chronic gastritis (~23 %) in our study. In peptic ulcer of stomach and duodenum, the relative number of infected patients dominated and amounted to ~93 % and 97 %, respectively. At the same time, chronic duodenitis in

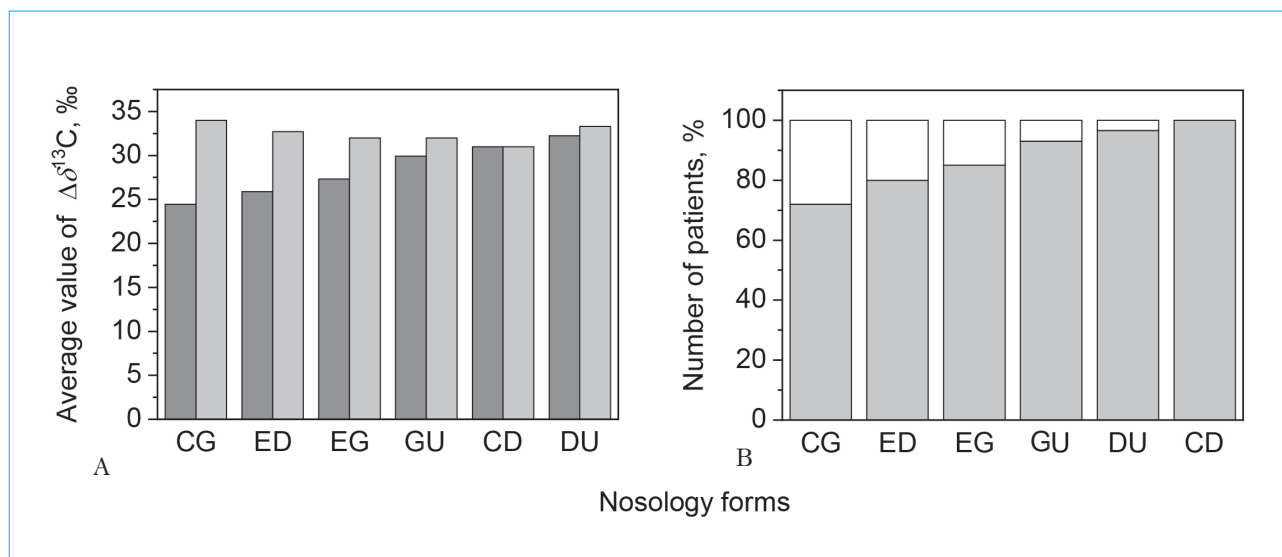


Fig. 3. Values of the laser ^{13}C -urea breath test for different nosological forms: A — average values of $\Delta\delta^{13}\text{C}$, taking into account both positive and negative test results (HP+, HP-) (light gray columns) in comparison with the average indicators of only positive outcomes (dark gray bars); B — the proportion of *H. pylori*-positive and negative patients with the corresponding nosological forms (CG — chronic gastritis, ED — erosive duodenitis, EG — erosive gastritis, GU — gastric ulcer, CD — chronic duodenitis, DU — duodenal ulcer)

100 % of cases was associated with the presence of infection.

Thus, the obtained experimental results show that taking into account the frequency of infection with the bacterium *H. pylori*, the average values of $\Delta\delta^{13}\text{C}$ in various nosological groups do not have significant differences. In addition, peptic ulcer disease in the vast majority of cases is associated with the presence of *H. pylori*, while in chronic gastritis and erosive duodenitis infection is not detected in some cases.

Method for comparing ^{13}C -urea breath test and histological data

From a methodological point of view, it is important to compare the results of histological examination and data obtained using ^{13}C -urea breath. These diagnostic approaches have significant methodological features and, in addition, fundamentally differ in the objects of observation. The urea breath test, unlike the histological examination, is not a direct method for detecting *H. pylori* bacteria. When used, the size of a colony of these bacteria is judged by the presence of the urease enzyme that they produce. In addition, the information provided by the UBT is averaged and summarized throughout the gastric mucosa. Its result is not sensitive to the localization of the *H. pylori* colony on the gastroduodenal mucosa. At the same time, histological examination uses pinpoint biopsies. Significantly different biopsy data from different parts of the gastrointestinal tract require their correct averaging. To conduct a histological assessment, a discrete (only 4 gradations) visual analog scale is used. Interpretation of the histological

study data depends on a number of subjective factors like qualifications and experience of the specialist conducting the analysis and the results interpretation. In this case, reliable quantification of *H. pylori* colony size is difficult. The ^{13}C -urea breath test gives a continuous series of values. They are obtained using analytical equipment giving quantitative result that is directly related to the amount of colonization. In turn, *H. pylori*-associated inflammation of the gastroduodenal mucosa can correlate with breath test results regardless of bacterial location. In this case, the UBT may reflect the relationship between colonization and the characteristics of the inflammatory process. In particular, in this way, the relationship between the urease activity of the *H. pylori* colony and the severity of changes in the mucous membrane in chronic gastritis and peptic ulcer can be established.

The total values of the results of the histological analysis of biopsy specimens collected from different sites of the gastroduodenal mucosa were used to compare the results of the ^{13}C -urea breath test and the morphological study. Since the breath test provides averaged and integral information about the *H. pylori* colony for the stomach and duodenum, such a comparison is more correct and allows taking into account the specifics of the compared methods. For each studied histological parameter (colonization, activity and severity of inflammation, degree of metaplasia and atrophy), an arithmetic sum was calculated for all parts of the gastrointestinal tract where biopsy specimens were taken and then was compared with the obtained value of $\Delta\delta^{13}\text{C}$.

The relationship of morphological assessment of the degree of colonization of the gastroduodenal mucosa with the results of ^{13}C -urea breath test

Figure 4 shows the dependence of the average values of $\Delta\delta^{13}\text{C}$ obtained before and after treatment on the total degree of *H. pylori* mucosa colonization in stomach body, antrum and duodenum (upper curve). One can see a monotonous increase in the results of the UBT depending on the total histological assessment of the degree of biopsy colonization by departments. Close to linear dependence of the result of UBT on the total degree of colonization is observed at low values of seeding. The interpolation curve drawn through the experimentally obtained points intersects the y-axis near zero values of $\Delta\delta^{13}\text{C}$. This result naturally confirms the fact that the decomposition of labeled ^{13}C -urea in the stomach is due exclusively to the urease activity of *H. pylori* bacteria. The slope of the curve at low colonization values is $\sim 13\text{ ‰/point}$. At high values of colonization, the growth rate of the curve decreases. The resulting dependence is generally logarithmic. This is confirmed by the almost linear dependence of the value of the exponent on the result of UBT on the indicator of total colonization (lower straight line on the graph). This type of dependence may be associated with a more intense and rapid depletion of the substrate (^{13}C -urea) with a high infection of the gastrointestinal tract with *H. pylori* bacteria.

Thus, a comparison of the results averaged over groups gives an unambiguous correspondence between the data of direct and indirect determination of the number of bacteria in the gastrointestinal tract. This indicates the legitimacy of a quantitative assessment of the degree of the mucosal *H. pylori* colonization using laser ^{13}C -UBT.

We also compared the results of ^{13}C -UBT and histological studies differentiated by topographic departments. Figure 5a shows the values of $\Delta\delta^{13}\text{C}$, averaged over groups of patients with the same degree of contamination of the mucosa in the antrum. An almost linear increase of the results of ^{13}C -UBT with the degree of colonization could be also observed in this case. The slope of the interpolation line is $\sim 13\text{ ‰/point}$. The value at which it intersects with the y-axis is close to zero $\Delta\delta^{13}\text{C}$, $\sim 4\text{ ‰}$.

Data on the stomach body are presented in Figure 5b. In this section, there is also a close to linear increase in the values of ^{13}C -UBT with the degree of colonization. The slope of the interpolation line is $\sim 11\text{ ‰/point}$. The level of intersection of the interpolation line with the y-axis is significantly higher than for the antrum and is $\sim 14\text{ ‰}$. The results of the study of the duodenum are presented in Figure 5c. The slope of the interpolation line in this case is

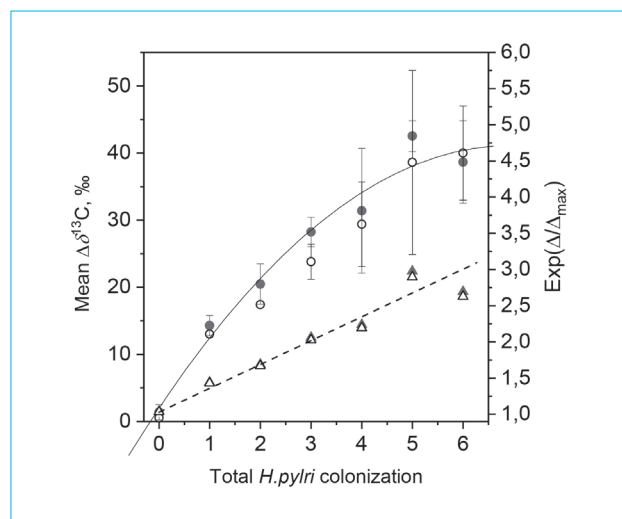


Fig. 4. The upper curve shows the average values of the ^{13}C -urease breath test depending on the total degree of *H. pylori* colonization in all departments: stomach body, antrum and duodenum, before (●) and after (○) eradication therapy. The lower straight line is the exponent from the upper experimental group of points, before (▲) and after (△) therapy

also $\sim 13\text{ ‰/point}$. The interpolation line intersects the y-axis at a level of $\sim 18\text{ ‰}$.

The analysis performed shows that at low degrees of colonization, the slopes of the interpolation lines for different parts of the gastrointestinal tract and for the total assessment in Figure 4 are close in magnitude and amount to $11\text{--}13\text{ ‰/point}$. This coincidence is associated with the method of histological assessment of colonization. It is known to be based on counting the number of bacteria that enter the microscope field of view when examining biopsy specimens of the gastroduodenal mucosa. Thus, due to a sufficiently large statistical sampling, a discrete morphological assessment is proportional with high accuracy to the concentration of bacteria in the biopsy specimen. This estimate is independent of where the tissue sample is taken from.

At the same time, as can be seen from Figure 5a-c, the level of intersection of the interpolation lines with the y-axis depends on the topography. This is explained by the fact that the contribution of bacteria colonizing different parts of the gastroduodenal zone to the result of the breath test is different.

Localization features of *H. pylori* bacteria in different parts of the stomach and duodenum in various nosological forms can be studied by comparing fairly representative statistical material of histological studies with the data of ^{13}C -urea breath test. Based on the results presented in Figures 4–5, a number of mathematical relationships can be established between the measured quantities.

The result of the breath test R_T can be represented as the sum of the contributions of the three areas

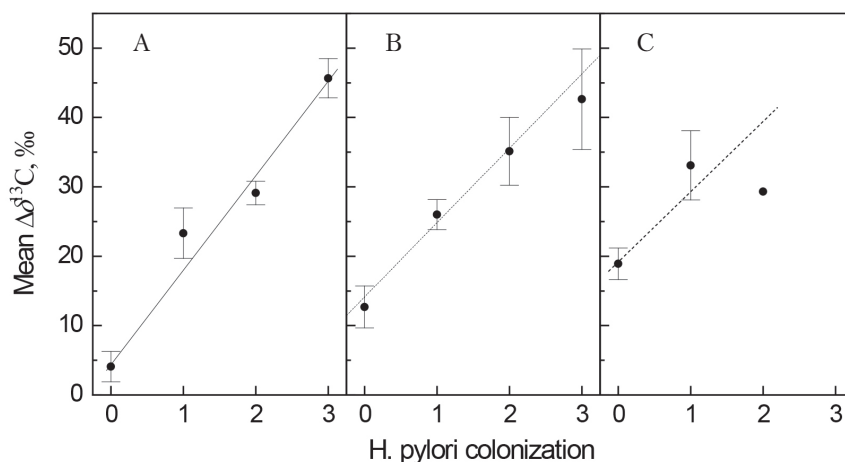


Fig. 5. Mean values of ^{13}C -UBT depending on the degree of *H. pylori* colonization in stomach antrum (A), in stomach body (B), and in the duodenum (C)

where the studied bacteria are located, including stomach body (R_B), stomach antrum (R_A) and duodenum (R_G). At the same time, the contribution of each of these departments to the result of UBT can be represented as the product of the concentration of bacteria (K_B , K_A , K_G) and the mucosal area (P_B , P_A , P_G) occupied by bacteria in the areas under consideration. This allows to represent the total result of the breath test R_T as a sum:

$$R_T = R_B + R_A + R_G = K_B \cdot P_B + K_A \cdot P_A + K_G \cdot P_G.$$

The value of the degree of colonization, obtained by the histological method, is a semi-quantitative assessment of the bacteria concentration on the mucosa. If one of the three regions under consideration has a histological score of zero colonization, then the two remaining regions will contribute to the breath test. These contributions can be determined by the level of intersection of the interpolation curves with the y-axis in the graphs of Figures 5A–C. Then, for the average values of the breath test $M(0)$ in groups of patients with a zero histological assessment of the colonization degree in each of the departments under consideration, we could obtain the following ratios relating these values to the contributions of the other two departments:

$$\begin{cases} R_T(K_B = 0) = K_A \cdot P_A + K_G \cdot P_G = M_B(0) \\ R_T(K_A = 0) = K_B \cdot P_B + K_G \cdot P_G = M_A(0) \\ R_T(K_G = 0) = K_B \cdot P_B + K_A \cdot P_A = M_G(0) \end{cases}$$

here: $M_B(0)$, $M_A(0)$, $M_G(0)$ – the average values of the breath test in groups of patients with a zero histological assessment of the degree of colonization in the stomach body, antrum and duodenum, respectively.

Taking into account that $R_B = K_B P_B$, $R_A = K_A P_A$, $R_G = K_G P_G$, we could obtain a system of three linear equations with three unknowns R_B , R_A and R_G :

$$\begin{cases} P_A + R_G = R_B \\ P_B + R_G = R_A \\ P_B + R_A = R_G \end{cases}$$

The ratios for the desired contributions of stomach body, stomach antrum and duodenum to the result of the breath test can be found by solving this system of equations:

$$\begin{aligned} P_B &= \frac{M_A + M_G - M_B}{2}; \\ P_A &= \frac{M_B + M_G - M_A}{2}; \\ P_G &= \frac{M_B + M_A - M_G}{2}. \end{aligned}$$

Substituting into these ratios the averaged values $\Delta\delta^{13}\text{C}$ for groups of patients with a zero histological assessment of the degree of colonization of stomach body, stomach antrum and duodenum, we get $R_B \approx 3.9 \pm 2.5$ ‰, $R_A \approx 14.1 \pm 1.5$ ‰ and $R_G \approx 0.1 \pm 2.0$ ‰. Thus, when averaged over all nosological forms, including gastric and duodenal ulcers, gastritis and duodenitis, the antrum of the stomach makes the most significant contribution to the result of the breath test. Its relative value is ~75 %. The contribution of the body of the stomach is ~23 % to the result of ^{13}C -UBT. The contribution of duodenum to the result of ^{13}C -UBT is difficult to determine from the available data due to the large scatter of the data. The determination error turns out to be greater than the determined value itself. Significant is the fact that the contribution of the duodenum to the result of the breath test is significantly less than the contributions of the antrum and body of the stomach. It can be as low as a few percent.

In this study, a fairly representative material was collected on the contributions of various areas of the gastroduodenal zone to the result of a breath test. This allows an analysis similar to that described above, separately for various nosological forms – gastric ulcer (GU), chronic gastritis (CG), erosive duodenitis (ED) and duodenal ulcer (DU). The average values of the breath test in groups with zero assessment of the degree of colonization in the body

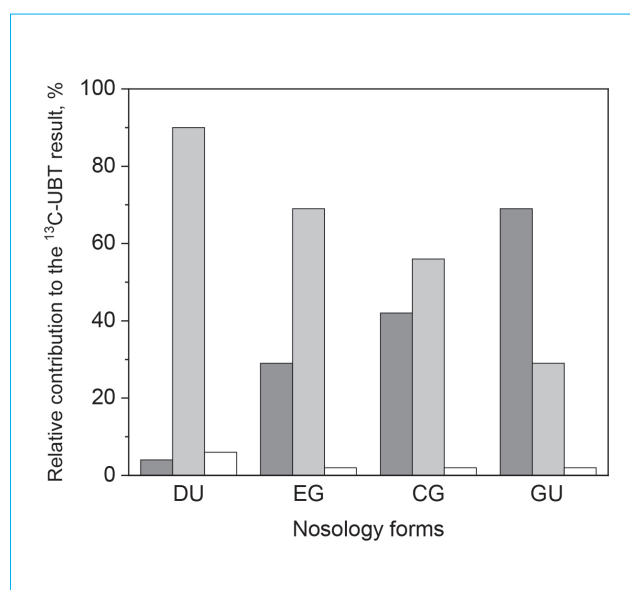


Fig. 6. Relative contributions of *H. pylori* urease activity in different parts of the gastroduodenal zone to the overall result of ^{13}C -UBT in various *H. pylori*-associated diseases (DU — duodenal ulcer, EG — erosive gastritis, CG — chronic gastritis, GU — gastric ulcer)

of the stomach (M_B), antrum (M_A) and duodenum (M_G) are presented in Table 1 for these nosological forms. Figure 6 shows the relative contributions of various departments to the values of the breath test, calculated according to the above formulas for different nosological forms. Since the result of the breath test reflects the number of bacteria that produce urease and are involved in the decomposition of ^{13}C -urea, the following conclusions can be drawn from these data:

- Only a small portion of all *H. pylori* bacteria is localized in the duodenum in all diseases. Their contribution to the result of ^{13}C -UBT in all nosological forms is no more than a few percent.
- With gastric ulcer, about 70 % of bacteria are localized in the body of the stomach, and with duodenal ulcer, more than 90 % of bacteria are located in the antrum of stomach. Thus, peptic ulcer disease is characterized by a higher concentration of *H. pylori* bacteria on the mucosa.

- The growth of the colony of *H. pylori* bacteria in the antrum is accompanied by a decrease in their content in the body of the stomach.

- Chronic gastritis is characterized by a close comparable number of bacteria in the body of the stomach and antrum.

- In erosive duodenitis the concentration of *H. pylori* bacteria in the antrum of the stomach is intermediate between gastritis and duodenal ulcer.

Thus, the data obtained show that gastric and duodenal ulcers, chronic gastritis and duodenitis have a specific pattern of predominant distribution of *H. pylori* bacteria along the gastroduodenal mucosa. The distribution plot of the ^{13}C -UBT data shown in Figure 6 quantitatively supports the suggestion that a high concentration of bacteria is more likely to lead to ulceration. At the same time, the dispersed distribution of infection over a large area, for example, the body of the stomach, in which the number of bacteria per unit area decreases, rather leads only to inflammation of the mucosa, i.e. chronic gastritis.

Comparison of morphological assessment of inflammation parameters and data of ^{13}C -urease breath test

It was also of interest to compare the results of the ^{13}C -UBT with the parameters of the inflammatory process caused by the presence of the infection. These parameters are also determined on the basis of histological studies of biopsy specimens. The presence of a relationship between inflammation parameters (severity and activity) and the number of bacteria *H. pylori* in the gastroduodenal area is less obvious. The process of inflammation has a complex picture depending on many factors. Among them are the pathogenicity of bacteria, the degree of the immune response of the human body, the degree of chronicity or severity of inflammation, and others.

A similar analysis was made by comparing the results of ^{13}C -UBT with the total assessment of the severity and activity of inflammation for the considered sections of the gastroduodenal zone characterizing the chronicity and severity of the inflammatory process. Such an analysis was carried

Table. Average values of $\Delta\delta^{13}\text{C}$ at zero degree of colonization in different parts of the gastrointestinal tract in patients with different nosological forms

^{13}C -UBT (K = 0)	Nosology form average, ‰	GU	CG	ED	DU
M_B	8.7	0	6.3	4.4	24.1
M_A	3.95	9.6	3.8	-0.3	2.7
M_G	18.42	23.1	15.5	11.6	23.5

GU - gastric ulcer; CG - chronic gastritis; ED - erosive duodenitis; DU - duodenitis ulcer

out on the primary survey data set for *H. pylori* positive patients. Figure 7F–D shows the dependence of the average values of the breath test on the total assessment of the activity and severity of inflammation of gastroduodenal mucosa, as well as the degree of its metaplasia and atrophy for stomach body, antrum and duodenum.

As can be seen from Fig. 7a, there is no clear relationship between the average values of ^{13}C -UBT and the total assessment of inflammation activity. The average UBT does not depend on the total assessment of the degree of neutrophilic infiltration of the gastroduodenal mucosa and, thus, on the activity of the inflammatory process. This can be explained by the different duration of *H. pylori* infection in the examined patients and the absence of active histological signs of acute inflammation in some of them. In addition, some patients could have *H. pylori* strains with mild pathogenic properties. Such bacteria may not cause active inflammation with corresponding mucosal morphological changes. As seen in Figure 7a, minimal histological signs of inflammation activity were obtained for the most *H. pylori*-positive patients. In *H. pylori*-negative patients, mucosal neutrophils were absent.

The total mucosa colonization characterized by the average value of $\Delta\delta^{13}\text{C}$ correlates quite strongly with the severity of mononuclear infiltration, Figure 7b. It is the higher the higher the assessment of the severity of inflammation and the stronger

the chronicity of the inflammatory process. In infected patients, the total scores for the severity of inflammation in most cases were not lower than 3 points. Thus, in the presence of *H. pylori* in the gastroduodenal zone, mononuclear infiltration will be observed in at least one of the sections of the gastrointestinal tract. This confirms the close relationship between the chronic inflammatory process in the stomach and duodenum and the presence of *H. pylori* infection. At the same time, weak and moderate mononuclear infiltration of the gastroduodenal mucosa persisted for the part of *H. pylori*-negative patients for whom the absence of infection was confirmed by both methods. This may be due to other causes of chronic inflammation of the mucosa including autoimmune nature.

Figure 7c shows the result of a comparison of the breath test data with histological signs of the degree of metaplasia of the gastric mucosa and duodenum. As can be seen, a monotonous decrease in the results of ^{13}C -UBT is observed with an increase in the degree of metaplasia. This can be explained by such changes in the morphological structure of tissues which lead to a decrease in the area of the gastroduodenal mucosa suitable for colonization by *H. pylori* bacteria. As a result, there is a decrease in the size of the bacterial colony in the gastrointestinal tract.

The average values of ^{13}C -UBT were also compared with the total assessment of atrophy of the gastroduodenal mucosa, Figure. 7d. As can be seen,

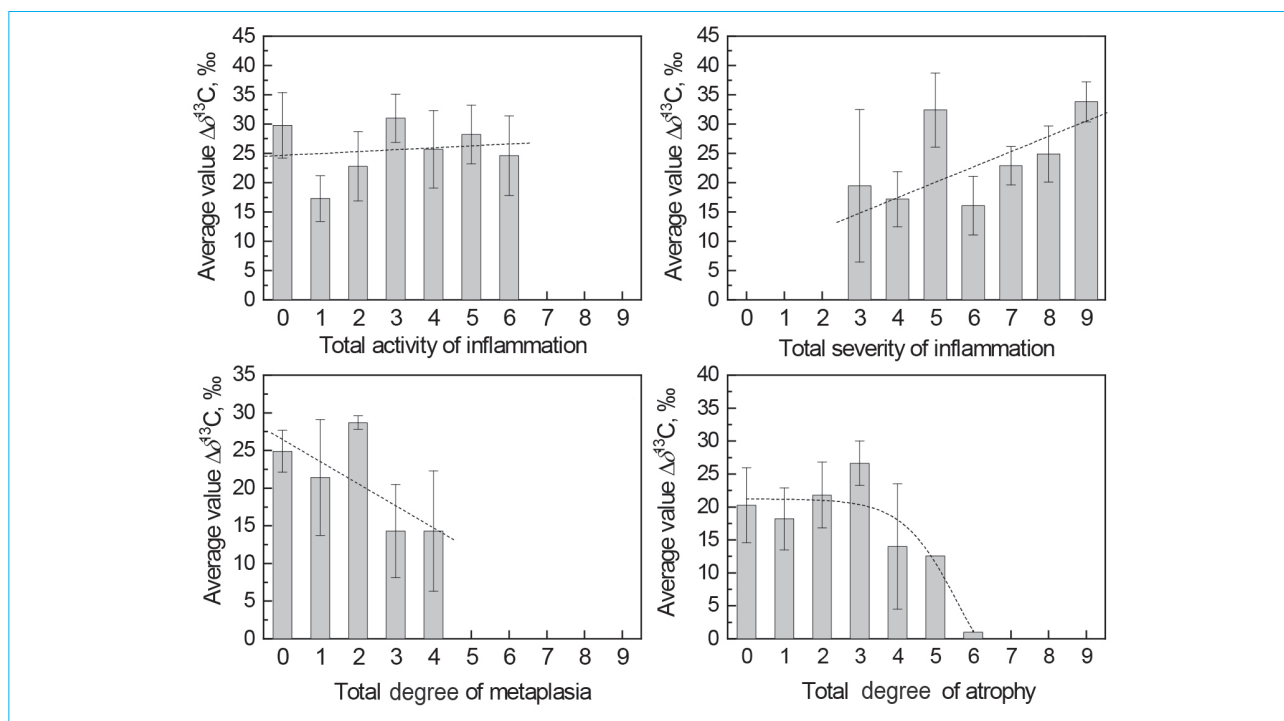


Fig. 7. Mean values of ^{13}C -UBT at various values of the total assessment: a – activity of inflammation of the gastroduodenal mucosa, b – severity of inflammation of the gastroduodenal mucosa, c – metaplasia of the gastroduodenal mucosa, d – atrophy of the gastroduodenal mucosa

at its low values of the degree of atrophy, the result of the breath test practically does not depend on this parameter. With the growth of atrophic changes, a decrease in the respiratory test is observed. However, due to the small amount of data on patients with severe atrophy, this conclusion is not sufficiently reliable. At the same time, the monotonous decrease in UBT values with an increase in the degree of atrophy can be explained by the fact that the atrophied mucosa, as a rule, is not colonized by *H. pylori* bacteria.

The discussion of the results

The main purpose of this work was to compare the data of the ^{13}C -UBT DLS with the data of histological analysis of biopsy specimens from different parts of the stomach and duodenum based on the sum of the scores of each parameter of the visual analogue scale.

Correspondence of the data of the direct histological method and indirect assessment of *H. pylori* infection with breath test demonstrates the possibility of quantitative assessment of the degree of infection based on the results of ^{13}C -UBT DLS.

Due to the complexity of the pathogenesis of the inflammatory process, it can be assumed that the relationship between the histological parameters of inflammation and the degree of *H. pylori* gastroduodenal zone will not be so obvious. However, it was possible to find a correlation between ^{13}C -UBT DLS parameters and the severity of inflammation, which was assessed by the degree of mononuclear infiltration of the mucosa (Figure 7b). Histological activity of gastritis/duodenitis in the majority of *H. pylori*-positive patients was low, and the correlation between the average values of ^{13}C -UBT DLS and the total assessment of the degree of mucosal neutrophilic infiltration, reflecting activity, was not detected (Figure 7a). In addition to the peculiarities of the development of the inflammatory response, this can also be explained by the different duration of the course of *H. pylori* infection in the examined patients, as well as by the different severity of the pathogenic properties of bacterial strains.

H. pylori, as a rule, does not colonize the atrophied gastric mucosa, so it was quite reasonable to expect a monotonous decrease in the values of $\Delta^{13}\text{C}$ as the severity of atrophy increases (Figure 7c). When comparing the parameters of the breath test with histological signs of the degree of mucosal metaplasia, no relationship was found. The lack of correlation between the values of $\Delta^{13}\text{C}$ and the severity of metaplasia indicates a greater autonomy of the metaplastic process and its indirect connection with *H. pylori* infection.

An analysis of the relationship between the results of ^{13}C -UBT DLS and individual nosological forms shows that in chronic gastritis the proportion

of infected patients is somewhat less (although the difference with other forms is not significant), and the average values of $\Delta^{13}\text{C}$ are lower ($p < 0.01$). The relationship with infection in chronic duodenitis and peptic ulcer was especially pronounced (Figure 6).

Mathematical processing of the results made it possible to reveal the features of the *H. pylori* distribution at various *H. pylori* — associated diseases. The results show that in gastric ulcer, approximately 70 % of bacteria are localized in the body of the stomach, and in duodenal ulcer, about 90 % of them are concentrated in the antrum. In chronic gastritis, the population of *H. pylori* in the body of the stomach and in the antrum is approximately equal, and erosive duodenitis is characterized by intermediate infection rates in the antrum between gastritis and duodenal ulcer. As the number of bacteria *H. pylori* in the antrum rises, their number in the body of the stomach decreases. The contribution of duodenal bacteria to the result of ^{13}C -UBT DLS for all studied *H. pylori* — associated diseases is relatively small (no more than a few percent), i.e. only a small proportion of bacteria is localized in this section.

Studies concerning an assessment of the relationship between the parameters of the ^{13}C -urease breath test and various parameters that mainly characterize the state of the stomach have been already undertaken. Interest in this problem arose at the very end of the 1990s [11], and similar studies continue to this day, but already taking into account factors that make it possible to minimize the probability of error. To this end, not only the method of the urease breath test was improved, but also more stringent criteria for selecting patients were applied, and the size of the groups increased. A study by Korean authors in a large group of patients ($n = 123$) showed a correlation between test parameters and the degree of infection of the gastric mucosa according to histology, as well as *H. pylori* on the surface of the gastric mucosa [12]. In another study in a small group of patients ($n = 25$), the relationship between test parameters and the activity of myeloperoxidase of neutrophils in the antral mucosa of the stomach (which indirectly reflects the activity of antral gastritis) was shown, while, however, no relationship was found with the degree of bacterial contamination, which was estimated by the number colonies [13]. In our work we used a highly informative method of laser spectroscopy and compared histological parameters with $\Delta\delta^{13}\text{C}$ in a fairly large group of patients ($n = 134$) using mathematical processing of the results.

In the surveyed population, some features of *H. pylori* infection are also shown. There is a gradual increase in $\Delta^{13}\text{C}$ in groups of patients from 15 to 35 years old (Figure 2), which, apparently, reflects the fact that primary infection and the development of associated diseases of the stomach

and duodenum occur at a young age. The decrease in *H. pylori* infection in older age groups can be explained by the more frequent development of atrophy with unfavorable conditions for *H. pylori* colonization.

Main conclusions

A direct linear relationship between the results of the breath test and the quantitative content of *H. pylori* on the surface of the gastric mucosa was

obtained. The parameters of laser ^{13}C -UBT clearly correlate with the degree of severity of mononuclear infiltration of the mucosa (the strength of the connection is 0.78). When comparing the average values of laser ^{13}C -UBT with the total assessment of the degree of mucosal neutrophilic infiltration, no significant relationship was found. With a slight severity of mucosal atrophy, the result of laser based ^{13}C -UBT practically does not change; there is a tendency to a decrease in the breath test with an increase in atrophic changes.

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