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# REGULATION OF THE SERUM GHRELIN LEVEL IN PATIENTS WITH TYPE 2 DIABETES MELLITUS INFECTED WITH HELICOBACTER PYLORI*

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Diabetes mellitus is a chronic metabolic disorder characterized by elevated plasma glucose. At the basis of the pathogenesis of the disease, two main mechanisms are distinguished, namely, the autoimmune destruction of ß-cells of the pancreas with subsequent insufficiency of insulin production, as well as endogenous resistance of body cells to insulin [1].

Diabetes mellitus is nothing but an epidemic of modern times, as according to statistics, the prevalence of diabetes has increased significantly over the last decade. Today, about 8.8% of the world's population has diabetes. If this trend continues, it is predicted that by 2040, about 693 million people aged 18-99, which is 9.9% of the world's population, will have diabetes mellitus [2].

*Helicobacter pylori* (HP) infection is another common pathology among the population. HP is a gram-negative bacterium that colonizes the human stomach and can lead to chronic gastritis, peptic ulcer disease, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma. This is a global infection that, according to the latest data, infects half of the world's population, and is more often observed in the population of economically and socially developing countries [3–5].

While conducting our research, the main question that arose was what link of pathogenesis connects these two global problems. And the answer ghrelin.

Ghrelin (GHR) has a wide range of physiological effects on all body systems and performs many functions in physiological and pathological conditions. It is the only known systemic signal that specifically promotes food intake and positive energy balance, and obesity by reducing fat oxidation, stimulates the functions of the gastrointestinal tract and acid secretion.

* The study was initiated by the authors within the search topic.

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GHR acts on exocrine and endocrine glands, provide the secretion of most hormones of the endocrine system [6, 7]. In addition, GHR positively affects glucose metabolism and insulin sensitivity, exhibits orexigenic and adipogenic effects that increase food intake and body weight, thus playing a pleiotropic role in modulating energy balance [8, 9].

GHR also plays a key role in the development of the pancreas and its secretory activity. In particular, it is able to support the viability and proliferation of ß-cells. In addition, GHR appears to exert an inhibitory effect on the pancreas [10].

The vast majority of studies show that HP suppresses the production and secretion of GHR. GHR production is restored after HP eradication, improving appetite and increasing BMI. When determining the concentration of circulating GHR in patients infected with HP, it was found that it is lower, compared to those who are not infected [11].

Moreover, the number of GHR-producing cells is lower in HR-infected individuals and in individuals with gastric lesions. In addition, the density of GHR-immunoreactive cells is higher in the oxyntic mucosa of HP-negative patients compared to HP-positive ones [12].

**The aim** — to assess the factors affecting serum ghrelin levels in patients with type 2 diabetes mellitus depending on *Helicobacter pylori* infection.

## MATERIALS AND METHODS

We examined 70 patients with non-insulin-dependent type 2 diabetes mellitus (T2DM, ICD-10-CM-Codes: E11) on the basis of the endocrinology department of the «A. Novak Transcarpathian Regional Clinical Hospital» (Uzhorod, Ukraine). The average age of the patients was 54 ± 3.4 years. Accordingly, 38 (54.2%) patients were female and 32 (45.8%) patients were male. The control group consisted of 20 practically healthy people, aged from 49 to 56 years. All patients were diagnosed with T2DM. The diagnosis of T2DM was established according to the recommendations of the American Diabetes Association (ADA, 2023 p.) [1], i. e. determination of the fasting plasma glucose level (FGL) value or the 2-h plasma glucose (2-h PG) level, which was carried out using the glucose-oxidizing method. The degree of diabetes compensation was assessed by the level of glycosylated hemoglobin ($HbA_{1c}$, %), which was determined using a chromogenic analysis on a Sysmex 560 device (Japan) using Siemens reagents. HP was determined using a test to detect HP antigens in feces (CITO TEST H. Pylori Ag, Pharmasco, Ukraine) and immunoenzymatic analysis of venous blood to determine the number of anti-HP antibodies. All patients had their serum GHR level determined by enzyme-linked immunosorbent assay using the Ray Biotech Human GHR ELISA Kit No 1.03930005306. The studies were conducted in compliance with the principles of the Helsinki Declaration of Human Rights, the Council of Europe Convention on Human Rights and Biomedicine, and the current legislation of Ukraine.

Statistical analysis. Data are presented as mean ($\bar{X}$) ± standard error of mean ($S_{\bar{X}}$). The Shapiro-Wilk test was used to test normality of data distribution. For multiple comparisons of data with a normal distribution, a parametric one-way analysis of variance (ANOVA) was performed and the Student's t-test was used. The relationship between the obtained characteristics was assessed using Pearson's linear correlation coefficient.

Linear regression and Fisher's test were also used. Values were considered statistically significant at $p < 0.05$.

## RESULTS AND THEIR DISCUSSION

Depending on the presence of HP in the patients, they are divided into two groups: group I with T2DM and HP-positive (HP+), which included 50 patients, and group II with T2DM and HP-negative (HP-) — 20 patients.

According to the data in Table 1, patients from group 1 (T2DM + HP+) showed a significantly higher level of the main parameters of carbohydrate metabolism, namely FGL, $HbA_{1c}$, I-HOMA and serum GHR level, which, in our opinion, indicates a certain role of HP in the

Table 1

**Average level of carbohydrate metabolism indicators depending on Helicobacter pylori infection**

| Indicator | Group I T2DM + HP+ n = 50 | Group II T2DM + HP- n = 20 | Control group n = 20 |
|---|---|---|---|
| Fasting blood glucose, mmol/L | 13.3 ± 2.9*^ | 6.9 ± 1.2^ | 4.03 ± 0.94 |
| Fasting insulin level, mIU/L | 24.4 ± 2.8^ | 17.9 ± 1.1^ | 10.05 ± 2.1 |
| $HbA_{1c}$, % | 8.9 ± 1.8*^ | 6.1 ± 0.5^ | 5.02 ± 0.11 |
| HOMA index of insulin resistance | 14.9 ± 1.3*^ | 5.6 ± 0.3^ | 1.05 ± 0.21 |
| Ghrelin, ng/mL | 159.1 ± 3.2*^ | 295.2 ± 0.9^ | 325.0 ± 5.76 |

*Notes:*
* statistically significant difference between the indicators of groups I and II ($p < 0.05$);
^ statistically significant difference between the indicators of groups I, II and control group ($p < 0.05$)

Table 2

**Factors affecting the serum level of ghrelin in group I**

Regression Summary for Dependent Variable: GHR
R = 0,96958976 R2 = 0,94010431 Adjusted R2 = 0,93755555
F(2,47) = 368,85 p < 0,00001 Std.Error of estimate: 0,79314

| | b* | Std. Err. | b | Std. Err. | t (47) | p-value |
|---|---|---|---|---|---|---|
| Intercept | | | 171.7892 | 0.542482 | 316.6725 | 0.0000001 |
| anti-HP IgG | −0.919756 | 0.077295 | −6.2415 | 0.524530 | −11.8993 | 0.0000001 |
| $HbA_{1c}$ | −0.055798 | 0.077295 | −0.0947 | 0.131188 | -0.7219 | 0.473943 |
| FGL | −4.67237 | 0.470744 | −5.0191 | 0.505678 | −9.92552 | 0.0000001 |
| I-HOMA | −3.74895 | 0.470744 | −2.2777 | 0.286003 | −7.96389 | 0.0000001 |

*Notes:*
FGL — fasting glucose level;
I-HOMA — HOMA index of insulin resistance.

regulation of not only carbohydrate metabolism, but also GHR.

To determine specific factors that affect the level of GHR in blood serum, regression analysis was performed in both groups (Tables 2, 3).

Analyzing Table 2, we can conclude that this model is reliable, since F = 368.85, i.e. Fisher's index, is greater than the estimated value of F = 2.47 ($p < 0.00001$). Taking into account this parameter, it can be argued that the regression linear polynomial is significant. The coefficient of determination by 94% determines the admissible dependent variable. According to Table 2, the level of anti-HP IgG, FGL and I-HOMA had reliable values. That is, with a decrease in the level of anti-HP IgG (−0.919756) by 1 unit, serum GHR will increase by 0,524530 ($p = 0.0000001$).

With a decrease in the FGL (−4.67237) by 1 unit, serum GHR will increase by 0.505678 ($p = 0.0000001$). In addition, with a decrease in the level of the I-HOMA (−3.74895) by 1 unit, serum GHR will increase by 0.286003 ($p = 0.0000001$).

This model is characterized by the following linear equation:

$$GHR = 316.6725 - 11.8993 \times \text{anti-HP IgG} - 9.92552 \times FGL - 7.96389 \times \text{I-HOMA}$$

When assessing the presence of dependence between FGL and anti-HP IgG, a reliable direct close correlation between these indicators was found ($r = 0.96875$; $p = 0.01$). That is, when the level of anti-HP IgG increases, there is an increase in glycemia (see Fig. 1).

When assessing the presence of dependence between the levels of insulin resistance

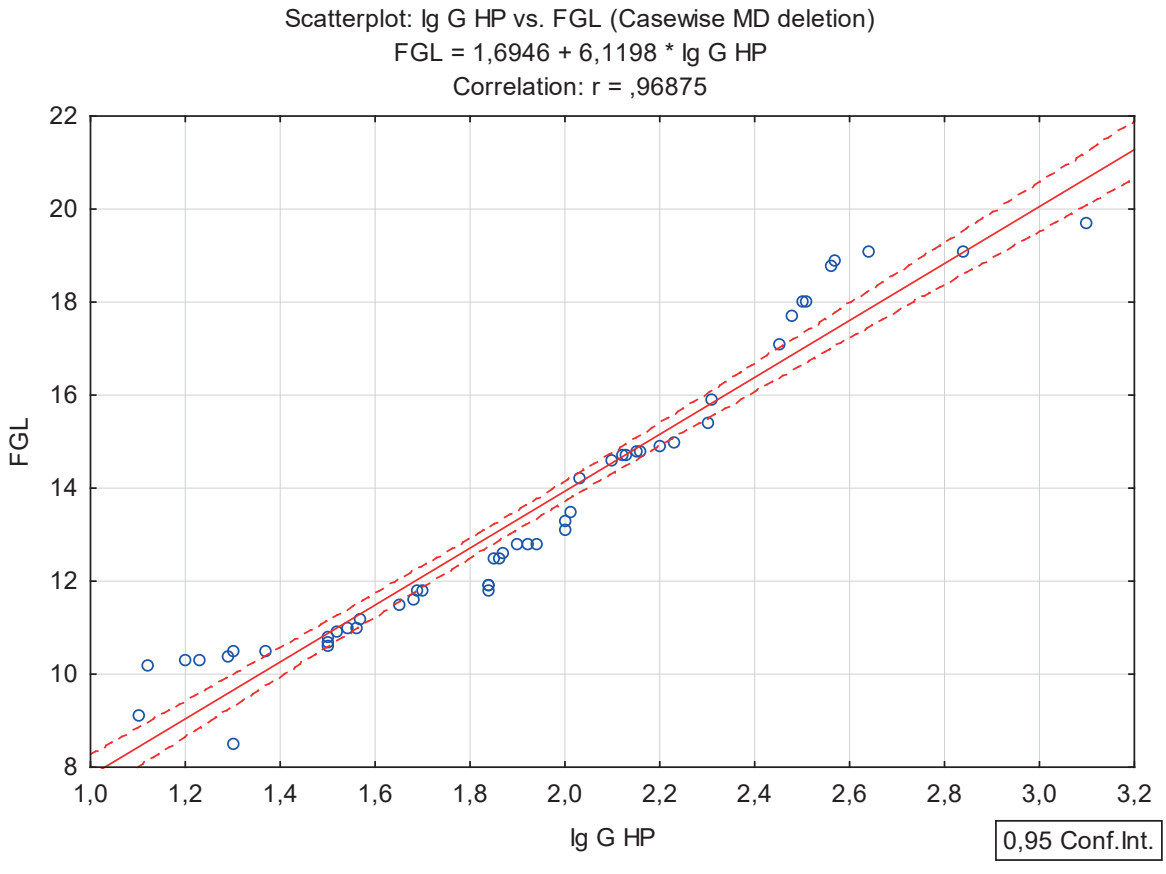


Fig. 1. The relationship between anti-HP IgG and fasting glucose level in group I.

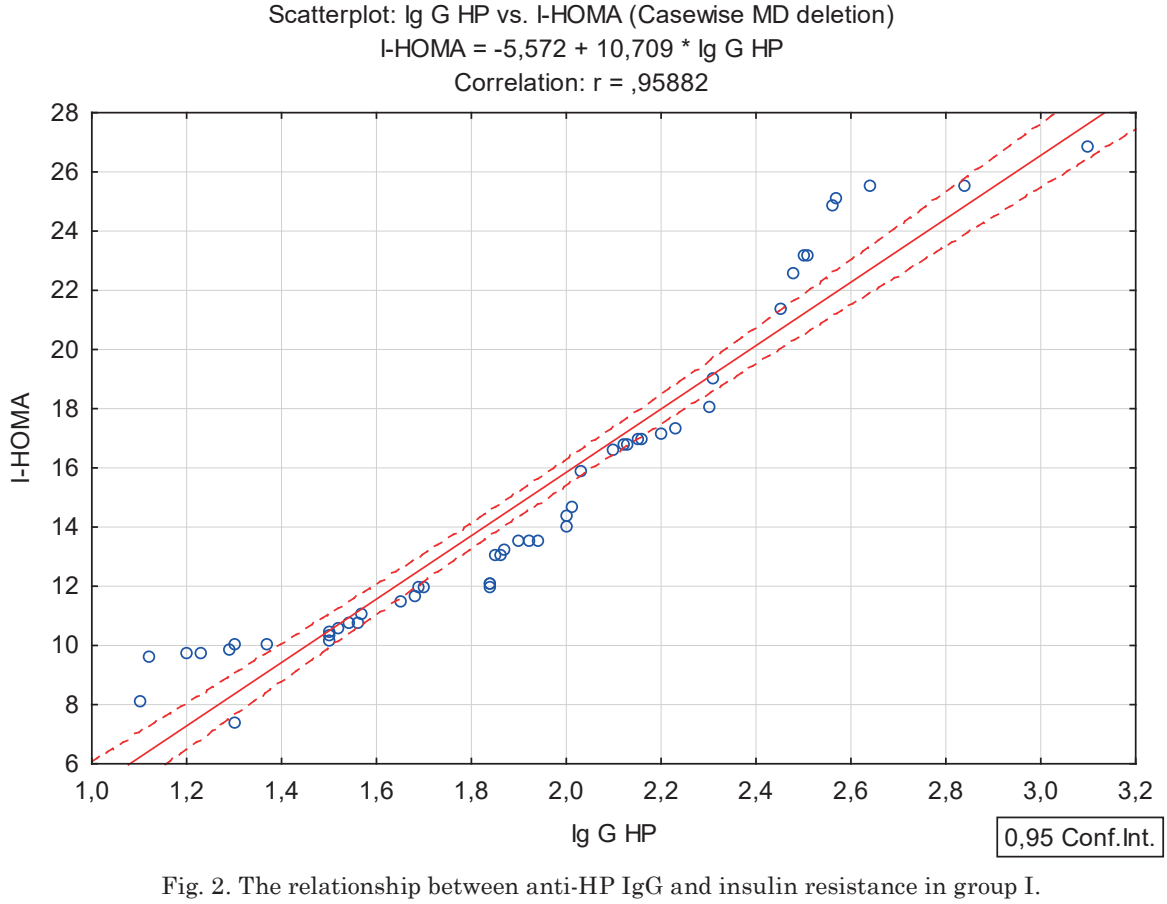


Fig. 2. The relationship between anti-HP IgG and insulin resistance in group I.

Table 3

**Factors affecting the serum level of ghrelin in group II**

Regression Summary for Dependent Variable: GHR
R = 0,97596814 R2 = 0,95251381 Adjusted R2 = 0,94692720
F(2,17) = 170,50 p < 0,000001 Std.Error of estimate: ,20256

| | b* | Std.Err. | b | Std.Err. | t(47) | p-value |
|---|---|---|---|---|---|---|
| Intercept | | | 292.0921 | 0.431664 | 616.6660 | 0.0000001 |
| anti-HP IgG | 0.335431 | 0.179498 | 1.3181 | 0.705357 | 1.8687 | 0.178993 |
| $HbA_{1c}$ | −0.650397 | 0.179498 | −0.4621 | 0.127541 | −3.6234 | 0.002099 |
| FGL | −1.74623 | 1.079145 | −1.3257 | 0.819288 | −1.6182 | 0.124031 |
| I-HOMA | −2.71500 | 1.079145 | −1.9291 | 0.766778 | −2.5159 | 0.022213 |

*Notes:*
FGL – fasting glucose level;
I-HOMA – HOMA index of insulin resistance.

and anti-HP IgG, a reliable direct close correlation between these indicators was found (r = 0.95882; p = 0.01). That is, with an increase in the level of anti-HP IgG, there is a deterioration of the sensitivity of cells to the action of insulin (see Fig. 2).

So, according to the obtained data, a reliable correlation between the key indicators of carbohydrate metabolism and HP infection was found. In addition to the effect on GHR, HP also affects the sensitivity to insulin, namely, increases insulin resistance, which is a negative prognostic factor for the course of T2DM.

Analyzing Table 3, we can conclude that this model is reliable, since F = 170.50, i.e. Fisher's index, is greater than the estimated value of F = 2.17 (p < 0.000001). Taking into account this parameter, it can be argued that the regression linear polynomial is significant. The coefficient of determination of 95.2% determines the admissible dependent variable. According to the Table 3, the level of $HbA_{1c}$ and the I-HOMA had reliable values. That is, with a decrease in the level of $HbA_{1c}$ (−0.650397) by 1 unit, serum GHR will increase by 0.127541 (p = 0.002). With a decrease in the level of the I-HOMA (−2.71500) by 1 unit, serum GHR will increase by 0.766778 (p = 0.02).

This model is characterized by the following linear equation:

$$GHR = 616.6660 - 3.6234 \times HbA_{1c} - 2.5159 \times \text{I-HOMA}$$

Analyzing and comparing groups I and II, it can be concluded that when infected with HP, the level of GHR depends not only on the metabolic changes' characteristic of T2DM, but also on the direct influence of the microorganism. In the group II, HP was not detected, and accordingly, the main factors that changed the level of GHR are $HbA_{1c}$ and insulin resistance.

Comparing our data with other scientific studies, we came to the conclusion that most sources showed the same research results, namely that the decrease in GHR level depends on human HP infection. Thus, in several studies, it was found that the concentration of GHR in the blood serum and the expression of its mRNA in the stomach tissue were associated with HP infection and gastritis and can be a criterion for assessing the state of health of the stomach tissue [13, 14].

Another study found an association of HP with metabolic disorders. Thus, HP disrupts glucose homeostasis, which leads to excess weight. Eradication of HP may improve carbohydrate metabolism, possibly due to increased GLP-1 (glucagon-like peptide-1) secretion [15].

One more study also confirmed the data we obtained, namely the participation of HP in the regulation of GHR secretion, as evidenced by a decrease in fasting GHR levels after HP eradication, which can be mediated by inflammatory responses in body tissues [16].

## CONCLUSIONS

Therefore, the level of serum ghrelin is significantly lower in patients with type 2 diabetes mellitus compared to individuals without diabetes, and is significantly dependent on the main indicators of carbohydrate metabolism. Infection with *Helicobacter pylori* leads to a decrease in the level of ghrelin in blood serum and an increase in insulin resistance and progressive decompensation of carbohydrate metabolism.

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# REGULATION OF THE SERUM GHRELIN LEVEL IN PATIENTS WITH TYPE 2 DIABETES MELLITUS INFECTED WITH HELICOBACTER PYLORI

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The article describes and analyzes the role of ghrelin in patients with type 2 diabetes mellitus (T2DM) infected with *Helicobacter pylori* (HP).

**The aim —** to assess the factors affecting serum ghrelin levels in patients with type 2 diabetes mellitus depending on *Helicobacter pylori* infection.

**Materials and methods.** 70 patients with non-insulin-dependent T2DM, according to the recommendations of the American Diabetes Association, aged 51 to 57 years, were included in the study. All patients underwent a determination of the level of glycosylated hemoglobin ($HbA_{1c}$), serum ghrelin (GHR), a fecal test for the determination of *Helicobacter pylori* antigen (HP) and anti-HP antibodies. Depending on the presence of HP in patients, they are divided into two groups: group I — HP-positive patients with T2DM (n = 50) and group II — HP-negative patients with T2DM (n = 20). The control group consisted of 20 practically healthy people, aged from 49 to 56 years.

**Results**. The obtained results indicate that patients with T2DM and HP+ have a significantly higher level of the main parameters of carbohydrate metabolism (fasting blood glucose, $HbA_{1c}$, HOMA index of insulin resistance (I-HOMA)), and GHR level is significantly lower in group 1 (T2DM, HP+) compared to group 2 (T2DM, HP-). The results of the regression analysis established that with a decrease in the level of anti-HP IgG (–0.919756) by 1 unit, serum ghrelin will increase by 0.524530 (p = 0.0000001), with a decrease in the level of fasting blood glucose (–4.67237) by 1 unit, GHR will increase by 0.505678 (p = 0.0000001), with a decrease in the level of glycosylated hemoglobin (–0.650397) by 1 unit, GHR will increase by 0.127541 (p = 0.002). With a decrease in the I-HOMA (–2.71500) by 1 unit in group 2, GHR will increase by 0.766778 (p = 0.02), with a decrease in the level of I-HOMA (–3.74895) by 1 unit in group 1, GHR will increase by 0.286003 (p = 0.0000001). A correlation was also established between fasting blood glucose and anti-HP IgG (r = 0.96875; p = 0.01); between the levels of insulin resistance and anti-HP IgG (r = 0.95882; p = 0.01).

**Conclusions.** The level of serum ghrelin is significantly lower in patients with type 2 diabetes mellitus compared to individuals without diabetes and is significantly dependent on the main indicators of carbohydrate metabolism. Infection with *Helicobacter pylori* leads to a decrease in the level of ghrelin in blood serum and an increase in insulin resistance and gradual decompensation of type 2 diabetes mellitus.

Keywords: type 2 diabetes mellitus, ghrelin, Helicobacter pylori.

# РЕГУЛЯЦІЯ СИРОВАТКОВОГО РІВНЯ ГРЕЛІНУ У ПАЦІЄНТІВ З ЦУКРОВИМ ДІАБЕТОМ 2 ТИПУ, ІНФІКОВАНИХ HELICOBACTER PYLORI

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В статті описано та проаналізовано роль греліну у пацієнтів з цукровим діабетом 2 типу, інфікованих *Helicobacter pylori*.

**Мета**. Оцінити фактори, що впливають на сироватковий рівень греліну у пацієнтів з цукровим діабетом 2 типу залежно від інфікування *Helicobacter pylori*.

**Матеріали та методи.** У дослідження було включено 70 пацієнтів з інсуліннезалежним ЦД 2 типу, згідно з рекомендаціями American Diabetes Association, віком від 51 до 57 років. Усім хворим було проведено визначення рівня глікозильованого гемоглобіну ($HbA_{1c}$), сироваткового греліну, фекальний тест на визначення антигену *Helicobacter pylori* (HP) та антитіла IgG до HP. Залежно від наявності у пацієнтів HP їх розділено на дві групи: група I — HP-позитивні хворі на ЦД 2 типу (n = 50) та група II — HP-негативні хворі на ЦД 2 типу (n = 20). Контрольну групу склали 20 практично здорових осіб віком від 49 до 56 років.

**Результати.** Отримані результати вказують на те, що у HP+ пацієнтів з ЦД 2 типу відзначається достовірно вищий рівень основних параметрів обміну вуглеводів (глікемії натще, HbA1c, індексу інсулінорезистентності I-HOMA), а рівень греліну достовірно нижчій у групі 1 (ЦД 2 типу, HP+) у порівнянні з групою 2 (ЦД 2 типу, HP-). Результати проведеного регресійного аналізу встановили, що зі зменшенням рівня IgG до HP (–0,919756) на 1 од. сироватковий грелін зростатиме на 0,524530 (p = 0,0000001), зі зменшенням рівня глюкози натще (–4,67237) на 1 од. сироватковий грелін зростатиме на 0,505678 (p = 0,0000001), зі зменшенням рівня $HbA_{1c}$ (–0,650397) на 1 од. сироватковий грелін зростатиме на 0,127541 (p = 0,002). Зі зменшенням рівня індексу I-HOMA (–2,71500) на 1 од. в групі 2 сироватковий грелін зростатиме на 0,766778 (p = 0,02), а зі зменшенням рівня I-HOMA (–3,74895) на 1 од. в групі 1 сироватковий грелін зростатиме на 0,286003 (p = 0,0000001). Також було встановлено кореляційну залежність між рівнями глюкози натще та IgG до HP (r = 0,96875; p = 0,01); між рівнями інсулінорезистентності та IgG до HP (r = 0,95882; p = 0,01).

**Висновки**. Рівень сироваткового греліну є достовірно нижчим у пацієнтів з цукровим діабетом 2 типу в порівнянні з особами без діабету і достовірно залежить від основних показників вуглеводного обміну. Інфікування *Helicobacter pylori* призводить до зменшення рівня греліну в сироватці крові і посилення інсулнорезистентності та поступової декомпенсації цукрового діабету 2 типу.

Ключові слова: цукровий діабет 2 типу, грелін, Helicobacter pylori.