THE ROLE OF GHRELIN AND LEPTIN IN THE FORMATION OF MORPHOLOGICAL CHANGES ESOPHAGUS OF PATIENTS WITH GASTRO-ESOPHAGEAL REFLUX DISEASE AGAINST TYPE 2 DIABETES

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Abstract

Type 2 diabetes mellitus (T2DM) is associated today with a non-infectious epidemic. Every year, the number of people suffering from this disease only increases. The comorbidity of pathology, namely gastroesophageal reflux disease (GERD) against the background of type 2 diabetes mellitus, is increasingly observed. Pathogenetic mechanisms of the progression of the latter in the combined course with type 2 DM are not sufficiently studied today, and data on the effect of adipokines on the morphological pattern of the esophagus in patients with type 2 DM are emerging.

The aim of this study is to study changes in the esophageal mucosa depending on the concentration of ghrelin and leptin in patients with comorbid GERD on the background of type 2 diabetes.

Materials and methods of the research. 120 patients were recruited for the study and divided into 3 groups and a control group. The 1st group – 60 patients with a combined course of GERD and type 2 DM, the 2nd group – 20 patients with GERD, and the 3rd group – 20 patients with isolated type 2 DM. The control group – 20 practically healthy people of the appropriate age.

The levels of ghrelin and leptin were determined by enzyme-linked immunosorbent assay (ELISA) on a Labline-90 analyzer (Austria).

Endoscopic examination of the upper parts of the gastrointestinal tract (GI) with targeted biopsy was performed with an esophagogastroduodenoscope with end optics «Olympus GIF Q 150-03» (manufactured by Olympus Europa SE & CO. KG, Japan).

The degree of complexity and the presence of reflux esophagitis were determined according to the Los Angeles classification and grading system of esophagitis of the Japanese Society of Esophagitis (JSDE), modified in 1999 [1].

Results. Correlation analysis of the relationship between ghrelin and leptin indicators showed a reliable, inverse, medium strength dependence ($r = -0.5531$; $p < 0.05$). Reliable maximum values of ghrelin were found in patients with non-erosive form of GERD, and minimum values of ghrelin were recorded in patients with reflux esophagitis stage C. When studying leptin indicators depending on the degree of esophagitis, the following data were obtained: the maximum values of leptin were found in patients with reflux – stage B esophagitis, and minimal – in patients with a non-erosive form of GERD. Regarding the reliability of the obtained data on leptin concentration, we did not find statistically significant differences in the studied groups ($p > 0.05$).

Conclusions. According to the results of the conducted research, we can say that changes in the concentration of ghrelin have an important diagnostic value in the focus of GERD against the background of type 2 diabetes. Decreased ghrelin levels have been associated with erosive forms of GERD in the setting of type 2 diabetes, which can lead to a significant deterioration in the patient's lifestyle. According to the data obtained during the study on the influence of leptin levels on changes in the mucous membrane of the esophagus, it was found that morphological changes in the esophagus did not depend on the concentration of leptin in the studied groups.

Keywords: type 2 diabetes, gastroesophageal reflux disease, ghrelin, leptin, morphological picture of the esophagus, reflux esophagitis.

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1. Introduction

Diabetes mellitus (DM) is one of the most important pathologies today, which leads to an increase in cases of disability and death of people. It affects people of different age groups, regardless of gender, country of residence and race. Research in recent years shows that the incidence of diabetes was 529 million people in 2021, and according to scientists, by 2050, this figure will be more than 1.31 billion cases. According to the World Health Organization (WHO), mortality from diabetes has increased by 3% in recent years [1, 2].

DM is classified into several types. First of all, attention should be paid to type 2 diabetes because a sedentary lifestyle, excessive nutrition, and excess weight are now considered possible predictors of development and cause such a prevalence. It is known that more than a third of all pathological conditions are a combination of the course of certain nosologies. Thus, more and more data are appearing about the combined course of disorders of the morphological picture of the esophagus, namely GERD, against the background of type 2 DM [3–5].

Regarding the pathogenetic mechanisms of disorders of the mucous membrane of the esophagus against the background of type 2 diabetes, it is known that complications inherent in patients with hyperglycemia can provoke certain changes in the body. Thus, diabetic neuropathy, gastroparesis, insulin resistance, changes in the concentration of adipocytokines and disruption of their functions due to changes in the sensitivity of receptors to these biologically active substances can be one of the more frequent causes of the occurrence and progression of GERD against the background of type 2 diabetes mellitus. Such adipokines as ghrelin and leptin are attracting increasing attention from scientists.

Regarding the mechanisms of the influence of leptin and ghrelin on the increase in body mass index (BMI), new data are emerging based on research in recent years. Thus, the work of Sitar-Tăut, A. V., Cozma, A., etc. (2021) indicated that the leptin/BMI ratio was significantly higher and the ghrelin/BMI ratio significantly lower in obese and diabetic patients. Obesity and DM may be associated with changes in the levels of adipokines (ghrelin, leptin), as the aforementioned changes are recorded in overweight people without existing DM [6].

There is an assumption that changes in the concentration of ghrelin against the background of violations of carbohydrate metabolism can provoke inflammatory reactions from the esophagus. Thus, in the works of the authors of recent years, we can observe lower levels of ghrelin in patients with GERD against the background of type 2 DM compared to higher levels in people without existing GERD. According to the literature search of the last 10 years, we observe a tendency to determine the main pathogenetic mechanisms of the formation of GERD against the background of type 2 DM, but the information is not exhaustive and requires further research in this direction [7–9].

Therefore, considering the above, we defined **the main aim**: to study the changes in the esophageal mucosa depending on the concentration of ghrelin and leptin in patients with comorbid GERD on the background of type 2 diabetes.

2. Materials and methods of the research

120 people were involved in the study, and they were divided into 3 groups and a control group. The 1st group included 60 patients with a combined course of GERD and type 2 DM, the 2nd group consisted of 20 patients with isolated GERD, and the 3rd group – 20 patients with type 2 DM. The control group included 20 practically healthy persons of the appropriate age. The patients were receiving inpatient treatment in the endocrinology and gastroenterology departments of the Communal Non-Commercial Enterprise of the Kharkiv Regional Council «Regional Clinical Hospital» in the period 2019 to 2021 years. The average age of patients in the 1st group was 57.60 ± 1.12 years. The age of patients in the 2nd group (isolated GERD) was 53.05 ± 3.68 years, in the group with type 2 diabetes (3rd group) – 54.60 ± 2.32 years. The control group included patients whose average age was 56.75 ± 2.54 years. The distribution of patients by gender and age was carried out in accordance with the standards of the WHO International Classification of Age Periods revised in 2015 [10]. Of the total number of patients, 44 (44 %) were men, and 56 (56 %) were women.

Bioethics. Protocol of the research report from the Committee on Bioethics of Kharkiv National Medical University (registration number 0118U000950, protocol No. 7 of September 11, 2018). Those patients who signed the informed consent for participation were involved in the study.

The diagnosis of type 2 diabetes was established following the classification of glycemic disorders (WHO, 1999), as well as following the unified clinical protocol of primary, secondary (specialised) and tertiary (highly specialised) medical care: type 2 diabetes (order of the Ministry of Health of Ukraine No. 1118 dated 12/21/2012).

The diagnosis of GERD was established based on patient anamnesis and upper endoscopy, in accordance with the current recommendations of the order of the Ministry of Health of Ukraine No. 943 dated October 31, 2013. European and American standards of diagnosis and treatment were considered [11–13].

Endoscopic examination of the upper part of the gastrointestinal tract with targeted biopsy (EPGDS) was performed with an esophagogastroduodenoscope with end optics «Olympus GIF Q 150-03» (manufactured by Olympus Europa SE & CO. KG, Japan). Patients were examined first in the upright position and then in the Trendelenburg position with tension to detect hiatal hernia. To increase the diagnostic value of EFGDS, the methods of chromoscopy and pinch biopsy of the mucous membrane of the esophagus were used. The study was conducted excluding increased intra-abdominal and intra-gastric pressure.

During endoscopic examinations, the presence and severity of reflux esophagitis were detected, changes in the colour of the gastric mucosa (erythema and its prevalence), and the presence of haemorrhages, erosions, and ulcers were considered. The degree of complexity and the presence of reflux esophagitis were determined according to the Los Angeles classification and the grading system of esophagitis of the Japanese Society of Esophagitis (JSDE) modified in 1999, presented in **Table 1** [14–17].

Table 1

Interpretation of the degree of severity and severity of reflux esophagitis

Degree of severity	Characteristics of changes in the mucous membrane of the esophagus
A	One (or more) defects of the esophageal mucosa less than 5 mm in length and width, limited by the tops of adjacent mucosal folds
B	One (or more) defects of the esophageal mucosa more than 5 mm in length and width, limited by the tops of adjacent mucosal folds
C	One (or more) esophageal mucosal defects extend beyond the tops of adjacent mucosal folds, occupying less than 75 % of the esophageal circumference (NOT circular)
D	Mucosal defects occupy more than 75 % of the circumference of the esophagus
Complications	Ulcer, bleeding, perforation, stricture, Barrett's esophagus, adenocarcinoma

The levels of adipocytokines ghrelin and leptin were determined by the ELISA method on the analyzer «Labline-90» (Austria), using a commercial test system and reagents Human GHRL (Grelin) ELISA Kit and Human LEP (Leptin) ELISA Kit manufactured by the company «ELABSCIENSE» (ELISA, USA) according to the instructions included in the set.

Statistical analysis was performed using the program StatTech v. 1.2.0, «Statistica 10», Excel 7.0. Comparison of indicators in groups was carried out using parametric (t – Student's test) statistics. The relationship between group indicators was evaluated using Pearson's correlation analysis (r – correlation coefficient). For all types of statistical significance analysis, differences were considered at $p < 0.05$.

3. Research results

During EPGDS in the group of combined courses of GERD and type 2 DM, the following changes of the esophageal mucosa were found in relation to the degree of esophagitis: 42 patients had an endoscopic picture without esophagitis, 13 patients had stage A esophagitis, and 5 patients had stage B esophagitis. **Fig. 1–3** show changes in the esophageal mucosa that were detected during EPGDS in this group.

We analysed the «ghrelin» indicator depending on the «degree of esophagitis» indicator in the group of the combined course of GERD and type 2 diabetes. The results are presented in **Table 2**.

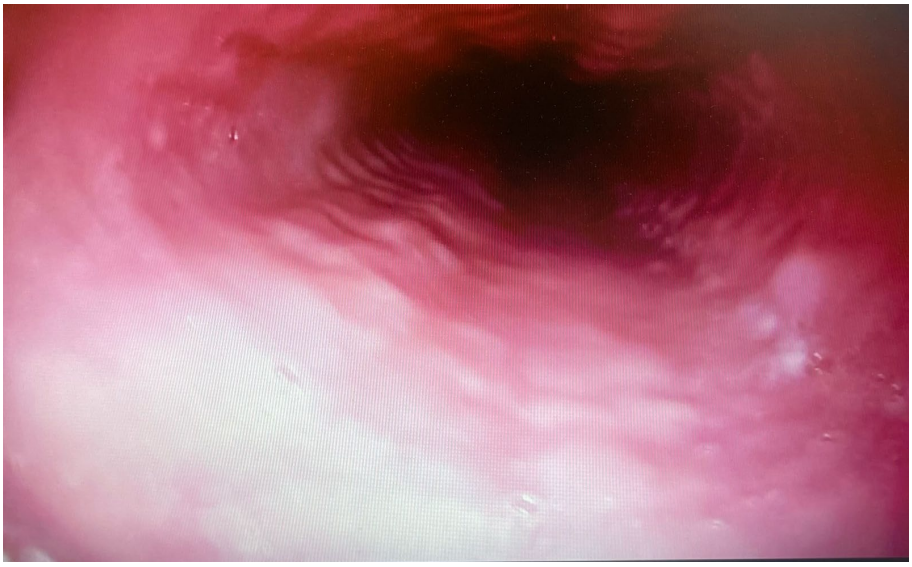

Fig. 1. Non-erosive form of GERD in patients with type 2 diabetes

Fig. 2. Reflux esophagitis, stage A in patients with type 2 diabetes

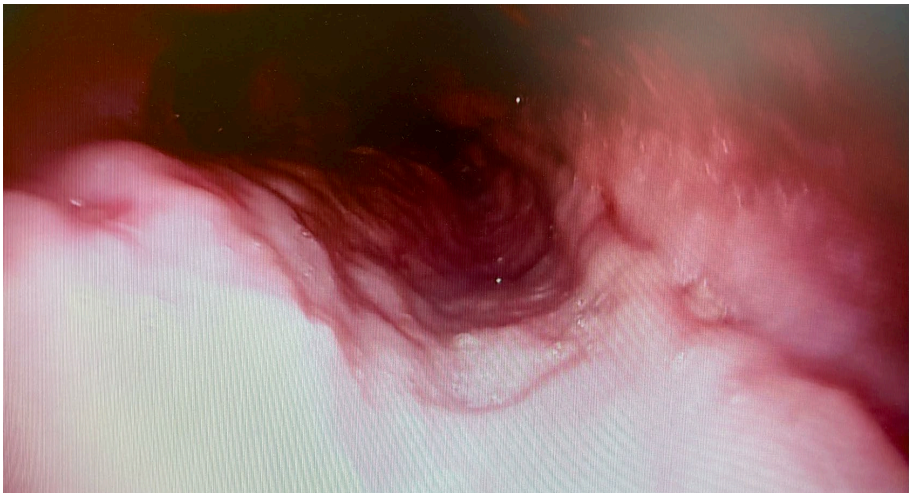

Fig. 3. Reflux esophagitis, stage B in patients with type 2 diabetes

Table 2
Analysis of the «ghrelin» indicator depending on the «degree of esophagitis» indicator

Indicator	Categories	Ghrelin, ng/ml			P
		Me	Q_1–Q_3	n	
Degree of esophagitis	Without esophagitis	0.92	0.73–1.20	42	$P_{0\text{-}A} = 0.085$
	Stage A	0.73	0.57–0.87	13	$P_{0\text{-}B} = *0.005$
	Stage B	0.56	0.50–0.67	5	$P_{A\text{-}B} = 0.068$

** – the differences are statistically significant ($p < 0.05$)*

Table 2 presents the average values of ghrelin with errors, which are compared with each other depending on the degree of esophagitis. Differences were found in all subgroups of the study (differences are statistically significant, $p < 0.05$). If we analyze the indicators of ghrelin depending on the degree of esophagitis, we can say that the maximum values of this adipokine were found in patients with GERD without esophagitis (**Fig. 4**).

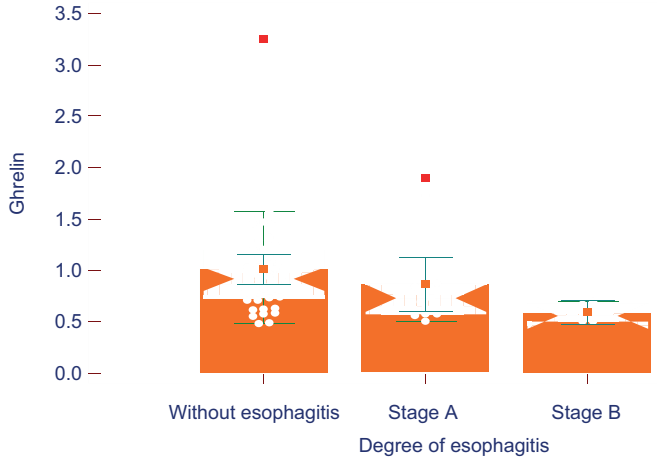

Fig. 4. Analysis of the «ghrelin» indicator depending on the degree of esophagitis

The following results were found when examining leptin levels depending on the degree of esophagitis in group 1 (**Table 3**).

Table 3
Analysis of the «leptin» indicator depending on the «degree of esophagitis» indicator

Indicator	Categories	Leptin, ng/ml			P
		Me	Q_1–Q_3	n	
Degree of esophagitis	Without esophagitis	16.75	10.05–22.72	42	$P_{0\text{-}A} = 0.394$
	Stage A	20.03	14.32–25.22	13	$P_{0\text{-}B} = 0.101$
	Stage B	25.15	19.03–30.13	5	$P_{A\text{-}B} = 0.430$

Table 3 presents the average values of leptin with errors, which are compared with each other depending on the degree of esophagitis. Differences were found in all subgroups of the study. If we analyze the leptin indicators depending on the degree of esophagitis, we can say that the maximum

leptin values were found in patients with reflux esophagitis, stage B, but we did not manage to find statistically significant results ($p > 0.05$) (**Fig. 5**).

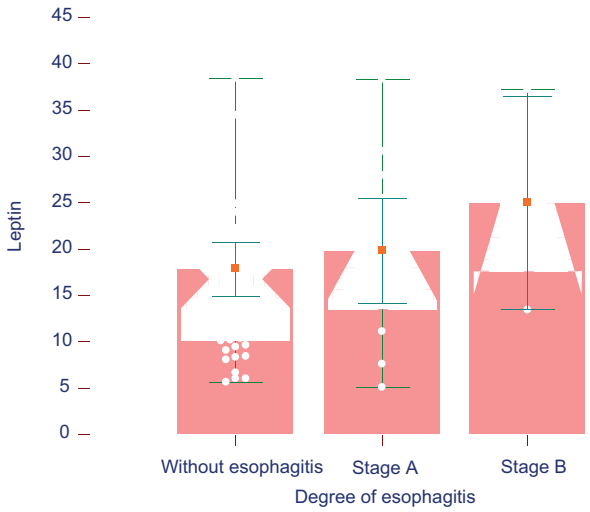

Fig. 5. Analysis of the «leptin» indicator depending on the degree of esophagitis

We conducted a correlation analysis of the relationship between the «ghrelin» and the «leptin» indicators. The results are presented in the **Fig. 6.**

According to the data in **Fig. 6**, it was found that the correlation between ghrelin and leptin was reliable, inverse, of medium strength ($r = -0.5531$; $p < 0.05$). That is, we can say that a decrease in leptin should be expected with an increase in the concentration of ghrelin.

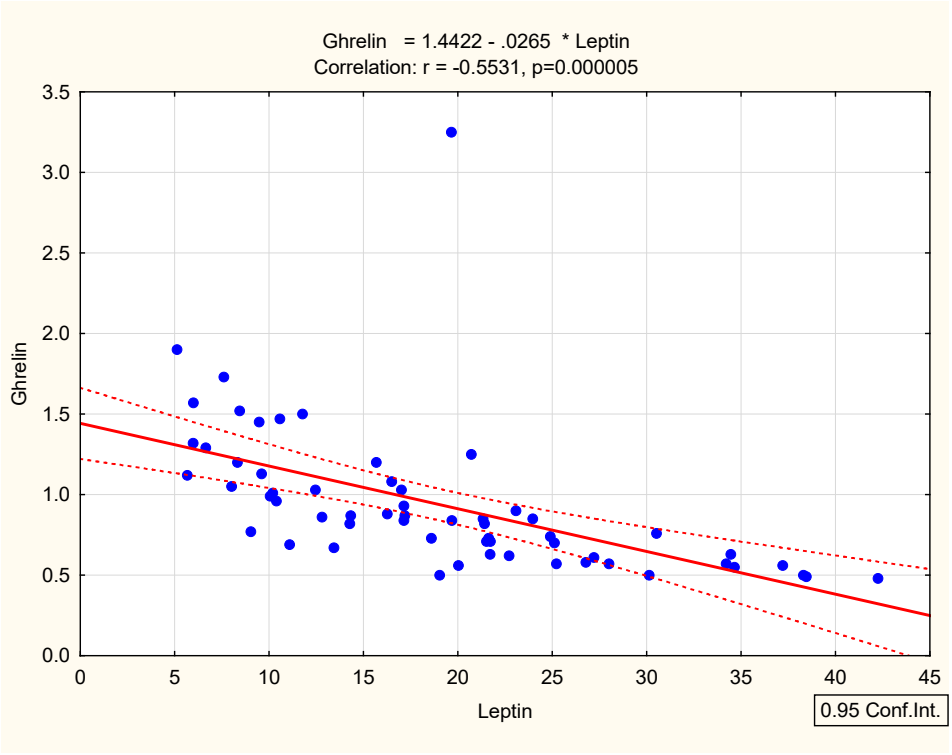

Fig. 6. Graph of the regression function characterizing the dependence of the «ghrelin» indicator on the «leptin» indicator

Evaluating the results of the study of the 2nd group (isolated GERD), the following changes in the esophageal mucosa were found in relation to the degree of esophagitis: 10 patients had an endoscopic picture without esophagitis, 7 patients had stage A esophagitis, 2 patients had stage B esophagitis, and 1 patient had stage B esophagitis. C. **Fig. 7** presents changes in the esophageal mucosa that were detected during EPGDS in this group. corresponding to stage C reflux esophagitis.

Fig. 7. Reflux esophagitis, stage C in a patient of the group with isolated GERD

Dependencies between the concentration of ghrelin and the degree of esophagitis, found in the study of patients with GERD, are presented in the **Table 4**.

Table 4
Analysis of the «ghrelin» indicator depending on the «degree of esophagitis» indicator

Indicator	Categories	Ghrelin, ng/ml			p
		Me	Q_1–Q_3	n	
Degree of esophagitis	Without esophagitis	1	1–1	10	0.032*
	Stage A	1	1–2	7	
	Stage B	5	4–6	2	
	Stage C	1	1–1	1	

** – the differences are statistically significant ($p < 0.05$)*

According to the presented table, when comparing the «ghrelin» indicator depending on the «degree of esophagitis» indicator, significant differences were established ($p = 0.032$) (*method: Kraskel-Wallis test)* (we can say that the maximum values of ghrelin were found in patients with a non-erosive form of GERD).

We conducted an analysis of the «leptin» indicator depending on the «degree of esophagitis» indicator. The results are presented in **Table 5**.

When evaluating the «leptin» indicator depending on the «degree of esophagitis» indicator, we failed to detect significant differences ($p = 0.271$, method: Kruskal-Wallis Test).

Table 5
Analysis of the «leptin» indicator depending on the «degree of esophagitis» indicator

Indicator	Categories	Leptin, ng/ml			p
		Me	Q_1–Q_3	n	
Degree of esophagitis	Without esophagitis	13	9–19	10	0.271
	Stage A	16	12–27	7	
	Stage B	27	26–28	2	
	Stage C	14	14–14	1	

4. Discussion of research results

A correlation analysis of the relationship between ghrelin and leptin indicators was conducted, which showed a reliable, inverse, medium-strength correlation dependence ($r = -0.5531$; $p < 0.05$). With an increase in the concentration of ghrelin, a decrease in leptin should be expected. When analyzing the studied groups, reliable maximum values of ghrelin were found in patients with a non-erosive form of GERD, and minimum values of ghrelin were recorded in patients with stage C reflux esophagitis. When studying leptin indicators depending on the degree of esophagitis, we did not find statistically significant differences in the studied groups. The obtained results are fully confirmed by other scientific works, which indicate a close relationship between changes in the concentration of ghrelin and the morphological pattern of the esophagus [18–20].

Study limitations. 120 people were included to participate in the study. Given that the number of patients may not be large enough to compare more observations, we can assume that the data obtained may vary slightly with further studies. It should be noted that the presence of concomitant pathology may have some influence on the final result in the studied groups.

Prospects for further research. In the future, it is planned to develop a prognostic model for the diagnosis of GERD on the background of type 2 diabetes and to optimize the therapeutic correction of the comorbid course of GERD and type 2 diabetes based on the study of the concentration of ghrelin and leptin.

5. Conclusions

Considering the great interest of researchers in ghrelin and the mechanisms of its influence on various links of pathogenesis in patients with GERD, we can say that there is a relationship between the concentration of this adipocytokine and changes in the morphological pattern of the esophagus against the background of type 2 DM. According to the results of the analysis of our work, it was found that elevated levels of ghrelin are observed in patients with minimal changes in the esophageal mucosa (non-erosive forms of GERD). In contrast, when its concentration is reduced, a tendency towards erosive forms of GERD against the background of type 2 diabetes was observed. So, we can say that the concentration of ghrelin is associated with changes in the esophagus and can be considered one of the markers for the early diagnosis of GERD in the future. Regarding leptin levels, depending on changes in the gastric mucosa, we did not find statistically significant results, so we can say that changes in leptin concentration were not associated with changes in the morphological pattern of the esophagus in the studied groups.

Conflict of interest

The authors declare that there is no conflict of interest in relation to this paper and the published research results, including the financial aspects of conducting the research, obtaining and using its results, and any non-financial personal relationships.

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Data availability

Data will be made available on reasonable request.

Use of artificial intelligence

The authors confirm they did not use artificial intelligence technologies when creating the current work.

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