Abstract. Background. In recent years, pathogenetic mechanisms underlying the association of gastroesophageal reflux disease (GERD) and metabolic syndrome have caused considerable interest among researchers. The purpose is to determine the features of cytokine balance, carbohydrate metabolism, leptin and ghrelin levels depending on the amount of visceral fat in patients with GERD. Materials and methods. Fifty-six patients with GERD were examined. According to the results of bioimpedance analysis, they were divided into the groups: 23 patients with a dangerous and 33 with a safe amount of visceral fat. The levels of tumor necrosis factor α, interleukin-6, interleukin-10, ghrelin and leptin were determined in the blood serum of all patients by the enzyme immunoassay method. Also, the levels of glucose, insulin were determined and the insulin resistance index (HOMA-IR) was calculated in patients with GERD. Results. In the group of GERD patients with a dangerous amount of visceral fat, an increase in the concentration of the pro-inflammatory cytokine interleukin-6 by 12 % was found with a simultaneous decrease in the level of the anti-inflammatory cytokine interleukin-10 by 22 % (p < 0.05), an increase in the HOMA-IR median by 1.3 times (p < 0.05) compared to the patients with a safe amount of visceral fat. The median level of ghrelin in patients with a dangerous visceral fat amount increased significantly, by 2.0 times (p < 0.05) compared to the control group. Correlation analysis showed an association between visceral fat and leptin (r = 0.37; p = 0.0008), interleukin-10 (r = −0.28; p = 0.0116), insulin (r = 0.41; p = 0.001), HOMA-IR (r = 0.48; p = 0.00001). Conclusions. In GERD patients, an increase in the amount of visceral fat was associated with a violation of the cytokine balance, carbohydrate metabolism, increased level of ghrelin. The determined statistically significant correlation between the amount of visceral fat and the level of leptin indicates a functional relationship between the body composition according to bioimpedance analysis and the hormonal activity of adipose tissue in patients with GERD.

Keywords: gastroesophageal reflux disease; visceral obesity; cytokines; insulin resistance; leptin; ghrelin

Introduction

All over the world, the number of people suffering from diseases of the gastrointestinal tract and hepatobiliary system and requiring specialized gastroenterological care is increasing annually [1]. Among digestive disorders, a special place belongs to gastroesophageal reflux disease (GERD), which affects approximately 20 % of the adult population in high-income countries [2]. In Ukraine, the statistical registration of GERD began in 2009, and, unfortunately, finished in 2017, so there were the latest data on the prevalence of GERD. However, there are isolated reports that GERD prevalence in Ukraine ranges from 7.6 to 23.9 % [3]. Among the well-known factors contributing to an increase in the prevalence of GERD, a growing number of overweight and obese people is of particular importance [4, 5].

At the same time, one of the most important problems of internal and family medicine is metabolic syndrome, which is often observed in patients with gastroenterological pathology [6, 7]. In a meta-analysis including 103,048 patients with GERD (15 studies), Fu S. et al. proved that risk fac-
tors for GERD among components of metabolic syndrome are abdominal obesity, hyperglycemia, hypertriglyceridemia and hypertension [8]. Moreover, A.A. Mironova, G.Yu. Tymoshenko noted in their study that patients with metabolic syndrome often have esophageal diseases (72 % of cases) manifested by non-erosive gastroesophageal reflux disease with extrapulmonary manifestations, insufficiency of gastric cardia, as well as esophageal hernias [6].

Among the components of metabolic syndrome, the most prognostically unfavorable factor today is visceral obesity — an increase in body weight due to abdominal fat [10]. Visceral fat (VF) is fundamentally different from subcutaneous fat, since it is actively involved in metabolic processes. More visceral fat is associated with more serious health consequences.

One of the methods for assessing the amount of visceral fat is the assessment of body composition using bioimpedance analysis, which represents measuring the bioelectric resistance of various body tissues when a safe (less than 90 A) electric current passes through the body, followed by computer processing [11].

It is known that the expression of inflammatory cytokine genes may be involved in the pathophysiology of various forms of GERD. Thus, in patients with erosive esophagitis, local expression of interleukin (IL) 18 was higher than in patients with non-erosive esophagitis [12]. The role of cytokine imbalance in obesity has been proven: a decrease in the content of serum cytokines, such as adiponectin and obestatin, was observed as well as increased content of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1β and interleukin-6 [13, 14].

Also, modern studies show that leptin affects glucose and lipid metabolism [15]. High concentrations of leptin are directly related to obesity and subsequent effects of metabolic diseases, such as insulin resistance, type 2 diabetes and cardiovascular diseases [16, 17]. At the same time, Abdelkader N.A. et al. found that weight gain and increased levels of the hormone leptin are associated with the clinical and endoscopic severity of GERD [18].

Thus, in recent years, pathogenetic mechanisms underlying the association of GERD and metabolic syndrome have generated considerable attention and interest among researchers.

The purpose was to determine the features of cytokine balance, carbohydrate metabolism, leptin and ghrelin levels depending on the amount of visceral fat in patients with GERD.

Material and methods

Fifty-six patients with GERD were examined: 16 women and 40 men aged 21 to 66 years, the median age was 39 years. They were treated at the department of stomach and duodenal diseases, dietetics and therapeutic nutrition of the SI "Institute of Gastroenterology of the NAMS of Ukraine". The control group consisted of 10 practically healthy individuals.

This study did not contradict the provisions of bioethics. It was approved by the Commission on Medical and Biological Ethics of the SI "Institute of Gastroenterology of the NAMS of Ukraine". The study complied with the Declaration of Helsinki; written informed consent was obtained from all patients.

All patients were evaluated for body structure using TANITA MC-780MA body composition scale analyzer (Japan). The mechanism of this apparatus is based on the bioimpedance analysis technique, which represents measuring the bioelectric resistance of various body tissues when a safe (less than 90 A) electric current passes through the body, followed by computer processing. Body mass index (BMI), waist/hip ratio, fat and muscle mass, amount of visceral fat were determined by this technique. According to the amount of visceral fat, two groups were identified: 23 patients with a dangerous amount of fat (more than 13 conventional units according to the manufacturer’s instructions) and 33 patients with a safe amount of fat (1—12 conventional units).

Serum levels of tumor necrosis factor α, IL-6 and leptin were determined by enzyme-linked immunosorbsent assay (ELISA) with appropriate reagent kits from Labor Diagnostika Nord (Germany). Serum IL-10 and ghrelin levels were assessed using appropriate test systems from Wuhan Fine Biotech (China). Insulin levels were determined by ELISA test systems from Monobind Inc. (USA). Insulin resistance was assessed using the HOMA-IR, calculated by the formula: HOMA-IR = fasting glucose (mmol/l) × fasting insulin (μOd/ml) / 22.5. ELISA was performed using a Stat Fax 303 Plus analyzer (USA), which measured optical density at 450 and 630 nm.

Statistical processing of the study results was performed with the Statistica 10 software package. Quantitative data are presented as median (Me) and interquartile interval (Q25; Q75). The probability of differences between quantitative values was evaluated using a non-parametric method (Mann-Whitney U-test), between qualitative data — with χ² criterion. When testing statistical hypotheses, the null hypothesis was discarded at a significance level less than 0.05. Spearman’s rank correlation coefficient was used to assess the degree of association between pairs of independent features.

Results

According to bioimpedance analysis, the median BMI in patients with GERD was 31.6 kg/m², with most patients having grade I obesity — 30 (53.6 %) (Table 1).

Increased waist/hip ratio (more than 0.85 in women and more than 1 in men) was observed in 9 women and 27 men. The median fat mass in the examined patients was 27.5 kg, while the median muscle mass was 63.3 kg.

A dangerous amount of VF, which can still be reduced by diet and physical exertion, was observed in 17 (73.9 %) out of 23 patients. A very dangerous amount of VF, requiring treatment by doctors — a nutritionist, endocrinologist, sometimes a bariatric surgeon, was diagnosed in 6 out of 23 (26.1 %) cases.

The analysis of pro-inflammatory cytokines found that in patients with a safe amount of VF, the median concentration of IL-6 was 5.1 times (p < 0.05) and TNF-α was 6.4 times higher (p < 0.05) compared to the control group (Table 2).

The median values of IL-6 and TNF-α in the serum of patients with dangerous amounts of VF were also 5.7 (p < 0.05) and 5.6 times (p < 0.05) higher, respectively, compared to the control values. It should be noted that in the group with a dangerous amount of VF, IL-6 concentration was 12 % higher than in patients with a safe amount of VF.
At the same time, the level of anti-inflammatory cytokine IL-10 in people with a dangerous amount of VF was 1.3 times lower (p < 0.05) compared to the group with a safe amount of VF. Correlation analysis showed an inverse relationship between IL-10 and VF amount (r = –0.28; p = 0.0116), HOMA-IR (r = –0.24; p = 0.005), with BMI (r = –0.29; p = 0.01), fat mass (r = –0.28; p = 0.001).

It was found that in patients with a safe amount of VF, the carbohydrate metabolism median did not differ statistically from the values in the control group (Table 1). Whereas patients with a dangerous amount of VF were characterized by an increase in insulin content by 1.6 times (p < 0.05) compared to the controls. An increase in the median of HOMA-IR by 1.4 times (p < 0.05) was found in patients with a dangerous amount of VF compared to the controls and by 1.3 times (p < 0.05) compared to the group with a safe amount of VF. The correlation of BMI with insulin content (r = 0.48; p = 0.001) and HOMA-IR (r = 0.55; p = 0.001) was demonstrated. In addition, correlations were found between the amount of VF and insulin (r = 0.41; p = 0.001), HOMA-IR (r = 0.48; p = 0.00001) (Fig. 2).

When assessing the level of adipocytokines depending on the amount of VF, an increase in the median content of ghrelin by 2.0 times (p < 0.05) was found in patients with both safe and dangerous amounts of VF compared to the control group (Fig. 3).

The leptin content in the studied patients ranged from 0.1 to 57.9 pg/ml. An increase in the level of this indicator above the norm was observed in 22 out of 23 (95.7%) patients with a dangerous amount of VF, which is 1.5 times higher than in the group with a safe amount of VF — 20 out of 33 (60.6%) cases (χ² = 7.11, p = 0.008). Correlation analysis
showed a functional relationship between leptin and BMI ($r = 0.62; \ p = 0.02$), HOMA-IR ($r = 0.39; \ p = 0.01$), VF amount ($r = 0.37; \ p = 0.0008$) (Fig. 4).

Therefore, in patients with GERD, an increase in the amount of visceral fat is associated with a violation of cytokine balance and carbohydrate metabolism, increased levels of ghrelin and leptin, which is confirmed by the revealed statistically significant correlations.

**Discussion**

In recent decades, GERD as a nosological form has been markedly increasing among the obese population [19, 20]. This fact encourages doctors and scientists to search for common pathogenetic mechanisms of disease development.

Along with this, the prevalence of obesity and metabolic syndrome is growing worldwide, acquiring the nature of an epidemic [21]. The progression of metabolic disorders contributes to the development of cardiovascular diseases, type 2 diabetes mellitus, and fatty liver disease, which significantly impairs quality of life, shortens life expectancy, and increases mortality at a young age [22, 23]. At the same time, the presence of visceral obesity is considered prognostically dangerous for the development and progression of cardiovascular and endocrine pathology [24]. In our study, visceral obesity was found in 23 of 56 (41.1%) patients with GERD.

According to modern concepts, the promotor of metabolic disorders is low-grade inflammation induced by adipocyte dysfunction in obesity. Metabolically active cells, such as adipocytes, secrete numerous anti-inflammatory cytokines and chemokines, adiponectin, leptin [25]. According to many authors, among inflammatory mediators, TNF-α and IL-6 play the main role in the pathogenesis of digestive diseases [26, 27]. However, in obese patients, the level of TNF-α is significantly higher than in non-obese people [28]. In our study, a significant increase in the serum levels of ghrelin, IL-6 and TNF-α in patients with GERD was found. It was not possible to reveal an increase in the level of IL-10 in the examined patients. This cytokine has an anti-inflammatory and modulating effect in inflammation and reduces the production of proinflammatory cytokines (TNF-α, IL-1β, IL-12) and the secretion of interferon gamma [27]. The absence of an inhibitory effect can lead to an imbalance in the inflammatory response and the predominance of pro-inflammatory factors, which causes a tissue damage [28].

Our study found that patients with dangerous levels of VF are characterized by an increase in insulin levels and the HOMA-IR, which correlated with BMI and the amount of VF. Insulin resistance is known as a trigger of visceral fat lipolysis and liver oxidation of free fatty acids, causing gluconeogenesis and fatty infiltration of hepatocytes [16]. Insulin resistance also leads to atherothrombotic changes in arteries, which results in changes of their elastic properties [17].

The median leptin concentration in the examined women and men increased along with an increase in BMI, depended on age, levels of VF, percentage of fat mass. Moreover, in women, the level of leptin was statistically significantly higher ($p < 0.05$) than in men. High leptin concentrations are directly related to obesity and the subsequent development of the effects of metabolic diseases, such as insulin resistance, type 2 diabetes, and cardiovascular disease [29]. Thus, the level of leptin increases with an increase in the mass of adipose tissue, its production in the subcutaneous fat cell is higher than in visceral fat depots [30]. Modern studies show that leptin also affects insulin sensitivity and lipid metabolism [29, 30]. Our study also revealed the association of leptin level with insulin resistance in patients with GERD. Insulin is also a regulator of leptin production. Prolonged hyperinsulinemia leads to an increase in plasma leptin concentration, while short-term hyperinsulinemia does not cause such a change [15].

Prospects for further research. The determination of the amount of visceral fat according to bioelectrical impedance analysis is considered a promising direction of non-invasive screening marker development in metabolic disorders in patients with GERD, which will improve the quality of medical care for Ukrainian population.

**Conclusions**

1. In the group of GERD patients with a dangerous amount of visceral fat, an increase in the concentration of the pro-inflammatory cytokine IL-6 by 12% was found as well as a simultaneous decrease in the level of the anti-inflammatory cytokine IL-10 by 22% ($p < 0.05$), an increase in the HOMA-IR median by 1.3 times ($p < 0.05$) compared to the group of patients with a safe amount of visceral fat.

2. A significant increase in the median ghrelin content by 2.0 times ($p < 0.05$) was detected in patients with GERD and a dangerous amount of visceral fat compared to the control group.
3. A statistically significant correlation between visceral fat amount and leptin level (r = 0.37; p = 0.0008) indicates a functional relationship of indicators of body composition according to bioelectrical impedance analysis with hormonal activity of adipose tissue in patients with GERD.

References


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Вплив вісцерального ожиріння на стан цитокінової та гормональної регуляції в пацієнтів із гастроезофагеальною рефлюксною хворобою

Резюме. Актуальність. Останніми роками розуміння значущу увагу до слідників привертають патогенетичні механізми, що лежать в основі асоціативного зв’язку гастроезофагеальної рефлюксеї з хвороби (ГЕРХ) і медитекольного синдрому. Мета: визначити особливості цитокінового балансу, вуглеводного обміну, рівня лептину та греліну залежно від кількості вісцерального жиру в пацієнтів із ГЕРХ. Матеріали та методи. Обстежено 56 осіб із ГЕРХ. За результатами біоімпедансного аналізу були сформовані вибірки: 23 пацієнти з небезпекою та 33 — з безпекою кількості вісцерального жиру в пацієнтів із ГЕРХ. В результаті кореляційного аналізу встановлено взаємозв’язки між кількістю вісцерального жиру і рівнем лептину (r = 0,37; р = 0,0008), інтерлейкіну-10 (r = 0,16; р = 0,0116), інсуліну (r = 0,41; р = 0,001), індексом HOMA-IR (r = 0,48; р = 0,00001). Висновки. Вісцеральне ожиріння, збільшення кількості вісцерального жиру асоціюється з порушенням цитокінового балансу та вуглеводного обміну, підвищенням рівня гепліну. Визначена статистично значуща кореляція між кількістю вісцерального жиру та рівнем лептину свідчить про функціональний зв’язок показників зміни складу тіла за даними біоімпедансометрії з гармоніальною активністю жирової тканини в пацієнтів із ГЕРХ. Ключові слова: гастроезофагеальна рефлюксенна хвороба; вісцеральне ожиріння; цитокіни; інсулінорезистентність; лептин; грелін

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