ORIGINAL ARTICLE

MANIFESTATIONS OF EXCESSIVE DAYTIME SLEEPINESS AND GHRELIN LEVEL IN CASE OF GASTROESOPHAGEAL REFLUX DISEASE IN PATIENTS WITH UNDIFFERENTIATED CONNECTIVE TISSUE DISEASE

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ABSTRACT

The aim of the study was to discover the interrelation between the severity of gastroesophageal reflux disease (GERD) symptoms, acid exposure time (AET), excessive daytime sleepiness (EDS) and the level of active blood plasma ghrelin in the patients with undifferentiated connective tissue disease (UCTD).

Materials and methods: The study included 120 patients with GERD. All the patients were divided in two groups: Group I - GERD was not accompanied by the signs of connective tissue disease (n=45) and Group II - GERD developed on the background of UCTD syndrome (n=75). Daily transnasal pH monitoring was performed to determine the nature of pathological refluxes. EDS was detected by The Epworth Sleepiness Scale. Active ghrelin in blood plasma samples was determined by ELISA.

Results: 80% of the patients of Group II and 35.48% of Group I suffered from EDS ($p<0.05$). The mean daily AET index was 5.48±0.4% in Group II and 6±0.2% in Group I, in the night hours mostly when patients were in the upright position. This phenomenon contributed to a deterioration of sleep quality and the appearance of EDS and was supported by a connection between AET and EDS ($r=+0.827$ for Group I and $r=+0.768$ for Group II). The mean De Meester index was higher in the patients of Group II (23.01±2.24 in Group I vs 31.08±2.4 in Group II; $p<0.05$).

Conclusions: GERD manifestations are strongly related to the level to AET and intensity of EDS. The EDS symptoms depend on circulating ghrelin level.

KEY WORDS: gastroesophageal reflux disease; undifferentiated connective tissue disease; ghrelin; excessive daytime sleepiness

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INTRODUCTION

Connective tissue diseases (CTD) are genetically determined conditions characterized by defects in fibrous structures and the ground substance of the connective tissue. CTD lead to the violation of organs and systems formation and have a progredient course. Undifferentiated connective tissue disease (UCTD) refers to unclassified systemic autoimmune pathologies having common clinical and serologic manifestations with certain connective tissue conditions (CTC) but do not respond to any of CTD classification criteria [1]. CTC are naturally heterogeneous, with clinical intersecting overlapping features, and therefore require careful differential diagnosis [2]. According to the recommendations [1, 2, 3], patients diagnosed with UCTD should be under constant, active follow-up in order to identify new signs of CTC.

The gastrointestinal tract is one of the systems that are most often involved in the pathological process on the background of UCTD. Esophageal diseases constitute about 80% of the total gastrointestinal pathology [4]. The important pathogenic component of gastroesophageal reflux disease (GERD) development is a functional abnormality of the lower esophageal sphincter (LES) [5]. In addition, dysplastic changes in the connective tissue create a premorbid background for the development of many pathological conditions and chronic diseases of whole organs. They affect the changes in the clinical picture, difficulties in early diagnosis, and more frequently promoted complications as a consequence. All of the above mentioned in patients with UCTD results in lower quality of life compared to the general population [6]. In addition, the daily discomfort, repeated visits to the doctor, the need for diagnostic interventions, and long-term but not always effective treatment are associated with high financial costs [7].

Despite the fact that heartburn is the main symptom of GERD, patients with concomitant UCTD may suffer from a number of other symptoms. According to UCTD criteria, such concomitant manifestations as Reynaud's Syndrome, arthralgia, photosensitization, unmotivated body weight loss, morning stiffness, dry mouth (xerostomia) and/or dry eye (xerophthalmia), dysphagia, recurrence of groundless fever, cutaneous lesions (rash), mouth ulcers, non-androgenic

alopecia, proximal muscular weakness may occur in such patients secondary to pathological gastroesophageal reflux [1]. All of them significantly complicate the diagnosis, the course of both the underlying and concomitant disease.

The intestinal hormone ghrelin modifies motility and the processes of GIT evacuation [8]. Furthermore, ghrelin affects the circadian rhythms of the body indirectly, via food intake [9]. The level of ghrelin in blood plasma fluctuates every day, with a peak during the day and decrease at night [10]. Its level also varies depending on the time of eating. Oin Wang et al. have experimentally demonstrated that ghrelin plays an important role in circadian rhythm disorders in case of fatty liver disease [11].

Ghrelin is one of the main hormones also responding to poor sleep and related disorders [12]. Experimental restriction of sleep duration was accompanied by an increase in ghrelin level, salt retention in the body, inflammatory markers, and promoted a decrease in insulin and leptin sensitivity [13, 14]. Since ghrelin is able to promote slow falling asleep and induce deep sleep [15], its level is likely to be able to affect sleepiness. At the same time, the knowledge related to the role of ghrelin in the case of GERD in general and in case of its combination with UCTD it is limited. Moreover, sleep and the circadian cycle play an essential role in the regulation of energy metabolism, affecting food intake, hunger, satiety, and regulates ghrelin secretion [16, 17]. Poor sleep quality may have a negative impact on metabolism since energy homeostasis is supported by the release of key hormones according to the daily circadian cycle. Experimental and clinical studies in adults have shown that both acute and chronic decrease in sleep lead to higher levels of ghrelin [13, 18]. In particular, Broussard et al. demonstrated that experimental sleep restriction in healthy young thin men led to increased ghrelin level [9]. McHill et al. found that circulating ghrelin level was affected by both circadian rhythms and the sleep-insomnia system [16]. However, they did not detect a reliable relationship between the changes in the circadian profile stability and subjective feelings of hunger.

Duration as well as the quality of sleep, affects the working efficiency during the day and the appearance of excessive daytime sleepiness. According to the scientific data about 80% of patients with GERD have particular nighttime symptoms, and every fourth person has the frequent ones that cause awakening and worsening of sleep [19]. Approximately 25% of the total population and 50% of patients with GERD inform about heartburn that disturbs sleep at night [19]. Many patients with GERD also experience short-term awakening during reflux, which contributes to sleep fragmentation and promotes low-quality sleep. Poor quality of sleep may increase sensitivity to intra-esophageal stimuli through brain-esophagus interaction [20]. Moreover, the LES relaxation allows the acid gastric contents enter the esophagus that could promote GERD symptoms. The number of spontaneous relaxations in their sleep increases in the patients with GERD, especially in rapid eye movement sleep, unlike in healthy individuals. According to the scientific data, rapid eye movement sleep is characterized by a decrease in the tonus of the skeletal muscle and sympathetic nervous system [20]. It has been scientifically confirmed that pathological GERD negatively affect sleep quality and, as a result, manifestations of excessive daytime sleepiness, however, these phenomena remain insufficiently studied in the patients with GERD associated with UCTD.

THE AIM

Based on the above we conclude that the issue of the GERD/sleep/ghrelin interrelation requires to be studied in more detail. This is why the aim of the study was to investigate the interrelation between the severity of GERD symptoms, acid exposure time (AET), excessive daytime sleepiness (EDS) and the level of active blood plasma ghrelin in the patients with undifferentiated connective tissue disease syndrome.

MATERIALS AND METHODS

The patients with GERD (in total n=120) treated at the Therapeutic Department of the University Clinic of Ivano-Frankivsk National Medical University (IFNMU) and the Therapeutic Department №2 of Ivano-Frankivsk Central City Clinical Hospital during 2016-2018 were examined upon the condition of the voluntary informed consent in accordance with the principles of bioethics and deontology. All the patients were divided in two groups: Group I - GERD was not accompanied by the signs of connective tissue disease (n=45) and Group II - GERD developed on the background of UCTD syndrome (n=75). UCTD syndrome was diagnosed according to the criteria recommended by M. Moska et al., A. Doria et al., T. I. Kadurina, L. M Abbakumova in the modification of T. Milkovskaya-Dimitrova. To assess the totality and severity of UCTD clinical manifestations, the Smolnova's scale was used. Symptoms of connective tissue dysplasia were divided into severe and mild. According to this scale, the total points up to 9 corresponded to the mild degree of CTD severity diagnosed in patients, from 10 to 16 – average degree of severity, from 17 and more – severe degree [1, 3]. GERD was diagnosed according to the criteria of the unified clinical protocol (the Order of the Ministry of Health of Ukraine from October 31, 2013 № 943). The criteria included typical for GERD complaints such as heartburn, dysphagia, regurgitation, esophagitis of different degrees according to the results of esophagogastroduodenoscopy (EGD), the data of daily pH monitoring. Los Angeles (LA) classification was used for the endoscopic assessment of the degree of esophagus lesion [18, 19]. In order to determine the nature of refluxes, daily pH was recorded in the lower third of the esophagus using transnasal pH Acidogastrograph with the registrar 1 pH-M (LLC "Start", Vinnitsa). Then patients completed a GERD – Q questionnaire for the symptoms of reflux disease. The information was also collected according to standard clinical examination. All patients were interviewed according to the Berlin questionnaire (1997) in order to evaluate the risk of obstructive sleep apnea syndrome (OSAS). [20]. Patients with a medium or high risk of OSAS were excluded from the study. The intensity of daytime sleepiness was detected using an adapted special scale for sleeping determination [22]. Briefly, the patients were asked to answer eight questions to assess their chances of falling asleep

while doing different activities. Their response was evaluated from 0 to 3 points depending on the probability of nodding. A person who scored 1-6 points had a normal sleep; 7-8 points meant moderate sleepiness; 9 points or more – a person was considered to have a significant daytime sleepiness .

Active ghrelin in blood plasma samples was determined by immuno-enzyme analysis with the use of The RayBio® Human Ghrelin Ensyme Immunoassay Kit (RayBiotech, Inc. USA). In order to preserve the ghrelin molecule stability, esterase activity was inhibited in all biological samples, following the recommendations of the test system manufacturer. Optical density reading was conducted with the use of immunoassay analyser (ImmunoChem-2100 Microplate Reader) at a wavelength of 450 nm.

The research was approved by the Bioethics Committee of Ivano-Frankivsk National Medical University and conducted according to the provisions of Council of Europe Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine (1997) and the principles of the World Medical Association (WMA) Declaration of Helsinki – Ethical Principles For Medical Research Involving Human Subjects (1964, revised by the 59th WMA General Assembly, Seoul, October 2008).

Statistical processing was performed using STATISTICA 7.0 and the statistical package of the Microsoft Excel 2016. The Student's-t test was used to confirm statistical differences ($p \leq 0.05$) between the indices in the groups. Correlation level between two selected parameters was determined using the Spearman's coefficient.

RESULTS AND DISCUSSION

The results of the ESS scale showed that 80% of the patients of Group II and 35.48% of the patients of Group I suffered from daytime sleepiness ($p<0.05$). Majority of the patients of Group II (n= 45, 60%; Fig. 1) suffered from moderate daytime sleepiness which was recorded more than twice compared to the patients with GERD without concomitant pathology ($p<0.05$). The signs of significant daytime sleepiness were present in 20% of the patients of Group II and in 8.88% of Group I ($p<0.05$).

According to the results of endoscopic examination, esophagitis severity in the patients of Group I was noted to correspond to LA-A, whereas it corresponded to LA-B more frequently in the examined patients of Group II. Stated differently, the endoscopic picture of the patients in Group II was characterized by severe hyperemia and persistent, frequently generalized mucosal edema.

According to the analysis of daily pH monitoring of the esophagus, total acid exposure time (AET) in the esophagus with the duration above normal was diagnosed in 57 (76%) patients of Group II and in 12 (28.8%) patients of Group I; the percentage of time with pH<4 in the upright position was noted in 36 (48%) patients of Group II and only in 8 (17.7%) patients of Group I; pH<4 in prone position constituted 60 (80%) vs. 10 (22.2%); the total duration of GER episodes lasting > 5 min was noted in 63 (84%) patients vs.13(28.8%) patients.

The number of refluxes per day constituted 57±8 (mean±SD) episodes with a total duration of 67±3 minutes in the patients of Group I, respectively, AET was 4.6±0.2% of the total monitoring period (Fig. 2A).

The number of acid refluxes constituted 79±6 episodes in the patients of Group II, total duration constituted 87±8 minutes, AET amounted 5.48±0.4% of the total monitoring period (Fig. 2B). Direct correlation between AET and EDS in both study groups was found: $r=+0.827$, $p<0.05$ in the GERD group and $r=+0.7684$, $p<0.05$ in the group of GERD associated with UCTD. The determination coefficient (R^2) indicated that the variation of the first indicator was caused by the variation of the second one by 68.4% among the patients of Group I and by 59.0% among the patients of Group II (Fig.2).

The mean De Meester index constituted 23.01±2.24 in the patients with GERD and 31.08±2.4 in the patients with GERD and UCTD combination ($p<0.05$). The percentage of time with pH<4 in the upright position and prone position increased from 10.2±0.4% and 5.8%±0.1 respectively in the patients of Group I, to 12.3%±0.05 and 7.4±0.1% in the patients with combined pathology ($p<0.01$). The duration of the longest pathological gastroesophageal reflux constituted 67±8 and 37±12 minutes among the patients of Groups II and I, respectively ($p<0.05$). The comparative pictures of the daily intra-esophageal pH-metry results typical for the examined patients are presented in Fig. 3.

In the course of the study, ghrelin indices were found to be significantly higher in the patients with GERD associated with UCTD in comparison with the patients with GERD without dysplasia signs (Fig.4.).

This index constituted 2413.18868±31.857 pg/ml on average in the patients of Group II and 471.499±14.472 ($p<0.05$) in the patients of Group I. The mean values of this index were 1.37 times higher in case of GERD and 7.03 times higher in case of its development secondary to UCTD compared to the control group.

A direct correlation between the manifestations of daytime sleepiness and the severity of pathological gastroesophageal reflux (GER) was also established. In particular, the occurrence of refluxes with pH<4 and a duration over 5 minutes correlated positively with the degree of excessive daytime sleepiness manifestation ($r=0.859$; $p<0.05$). Concurrently, we observed a direct linear dependence between the occurrence of refluxes with pH<4 and a duration over 5 minutes and the level of ghrelin in blood plasma – $r=0.659$, $p<0.05$ in the patients of Group I; the occurrence of refluxes with pH<4 and a duration over 5 minutes and the concentration of ghrelin in blood plasma – $r=0.786$, $p<0.05$- in Group II.

According to the correlation and regression analyses, a strong, direct, reliable correlation ($r=+0.86$; $p<0.05$) between the severity of excessive daytime sleepiness and the level of ghrelin was noted in the patients of Group I, and the determination coefficient (R^2) indicated that the variation of the first indicator was caused by a variation of the second one by 73.9%. The indices constituted +0.80, $p<0.05$ and 64.1% in the patients of Group II, respectively. The graphical representation of the analysis results is shown in Fig. 5A) and 5B).

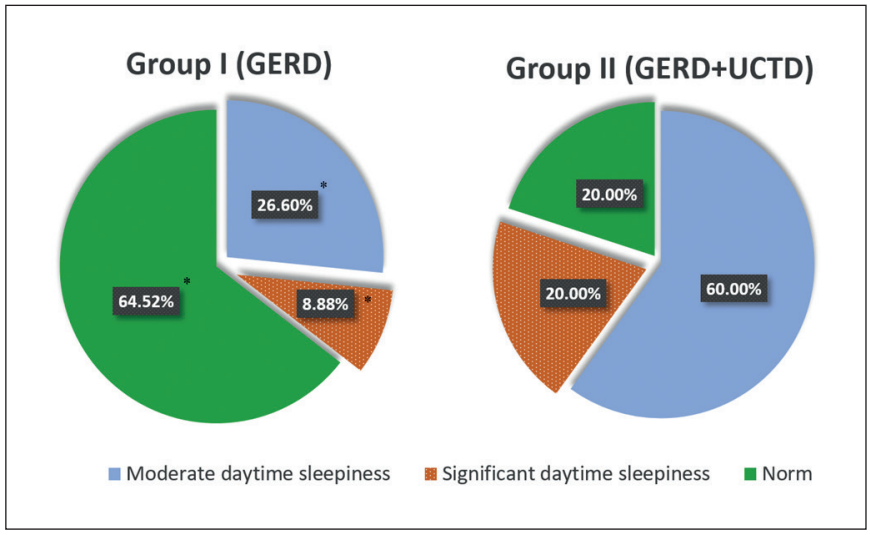

Fig. 1. The distribution of the patients according to the Epworth Sleepiness Scale. Significant differences ($p<0.05$) were found when comparing the reliable data between the research groups.

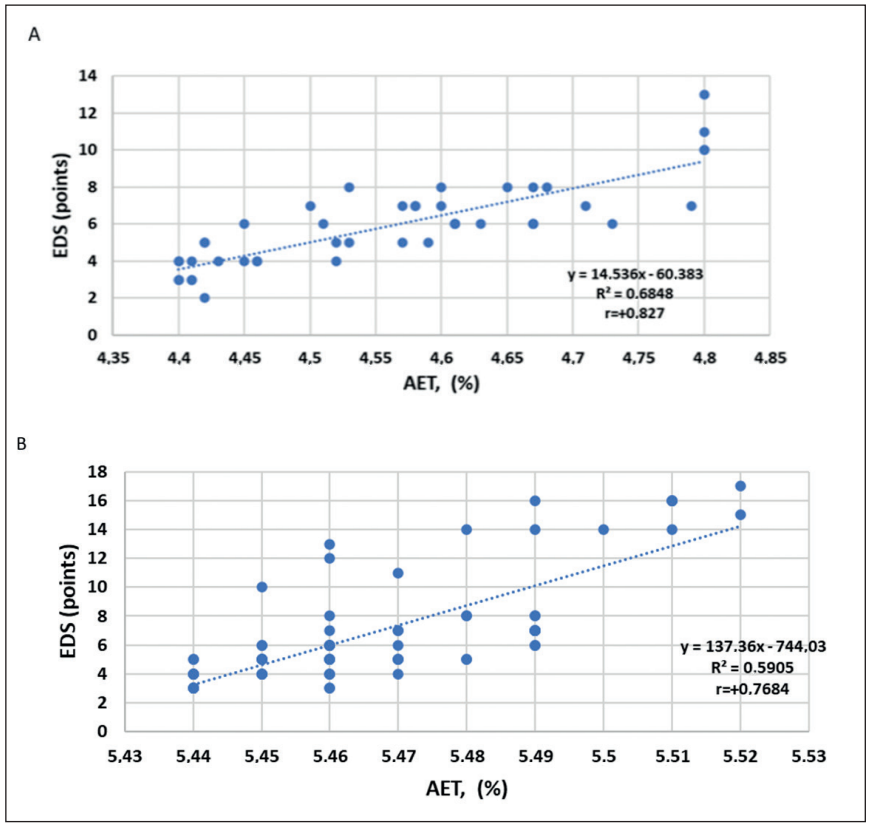

Fig. 2. Relation between EDS index on The Epworth Sleepiness Scale and AET. A) in the patients with GERD. Correlation field and regression line (with equation) describing it ($r = +0.827$; $p<0.05$); B) in the patients with GERD associated with UCTD. Correlation field and regression line (with equation) describing it ($r=+0.768$; $p<0.05$); R^2 is the determination coefficient.

DISCUSSION

According to the conducted research, the presence of UCTD complicated the condition of the patients with GERD. Severe sleep disturbances were proved to occur in the patients with GERD associated with UCTD leading to excessive daytime sleepiness. The data were compliant with provisions of National Sleep Foundation (NSF), according to which, people who experience heartburn at night are more likely to suffer from insomnia and excessive daytime sleepiness than those who do not have heartburn at night.

The patients with GERD associated with UCTD complained of superficial sleep with frequent awakening significantly more often ($p<0.05$). According to the analysis of daily pH monitoring of the esophagus, AET index constituted 5.48±0.4% in the patients with combined pathology and 6±0.2% in the patients with GERD mainly on account of the night hours when the patients were in prone position. This phenomenon contributed to the decrease in sleep quality and the occurrence of excessive daytime sleepiness, as it was evidenced by the direct strong relationship between AET and EDS in both research groups: $r=+0.827$, $p<0.05$ in the group of patients with GERD and $r=+0.7684$, $p<0.05$ in the group of patients with GERD associated with UCTD. The data were compliant with the scientific data of Yamasaki T. with co-authors. According to them, sleep deficiency increased the number of pathological GER, contributed to the increase

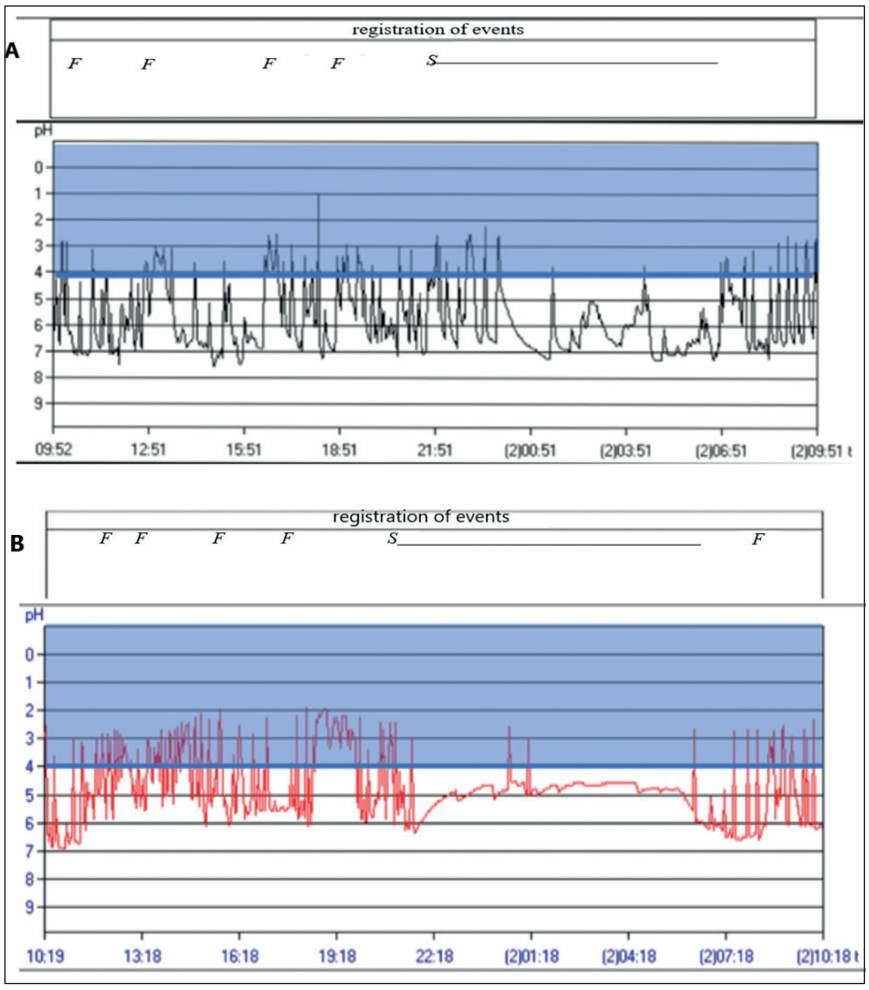

Fig. 3. Typical daily intra-esophageal pH-metry of a patient with GERD (A) and patient with GERD associated with UCTD (B). Conventional symbol: F- food; S-sleep.

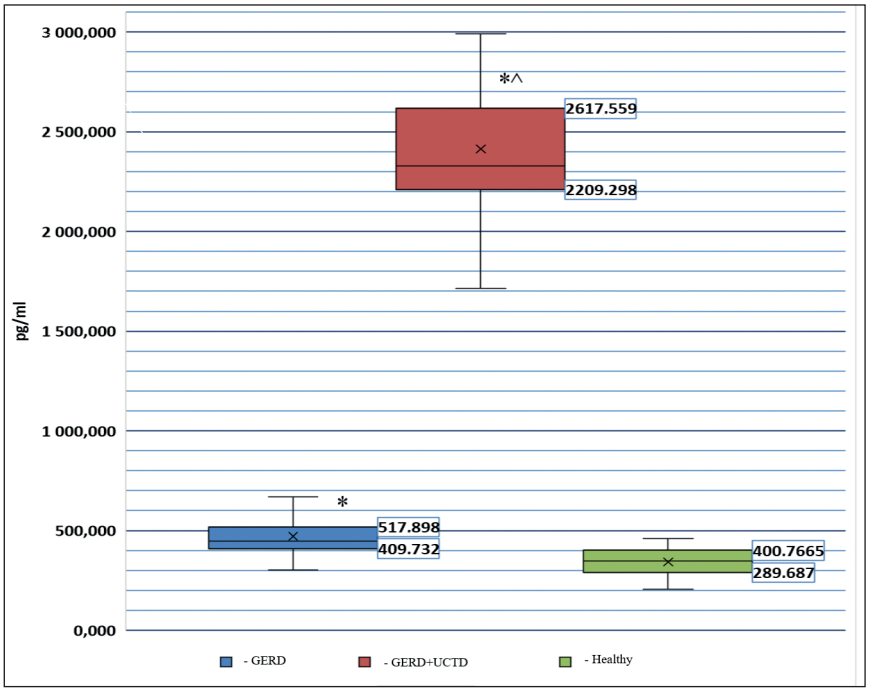

Fig.4. Ghrelin indices in the blood plasma of the patients with GERD in combination with UCTD and GERD without concomitant pathology. Mo - mode, Me - median, and interquartile range: lower - higher quartile (LQ-HQ) were used for non-normal distribution.
* – ($p<0.05$) the data are reliable in comparison with the indices of healthy individuals;
^ – ($p<0.05$) the data are reliable in comparison between the research groups.

in the duration of acid exposure from the mucosa of the lower third of the esophagus both in the healthy individuals of the control group and in patients with GERD on the basis of daily pH-metry [21]. Meanwhile, Navarro-Solera M. and co-authors found no relation between sleep duration and ghrelin level [23].

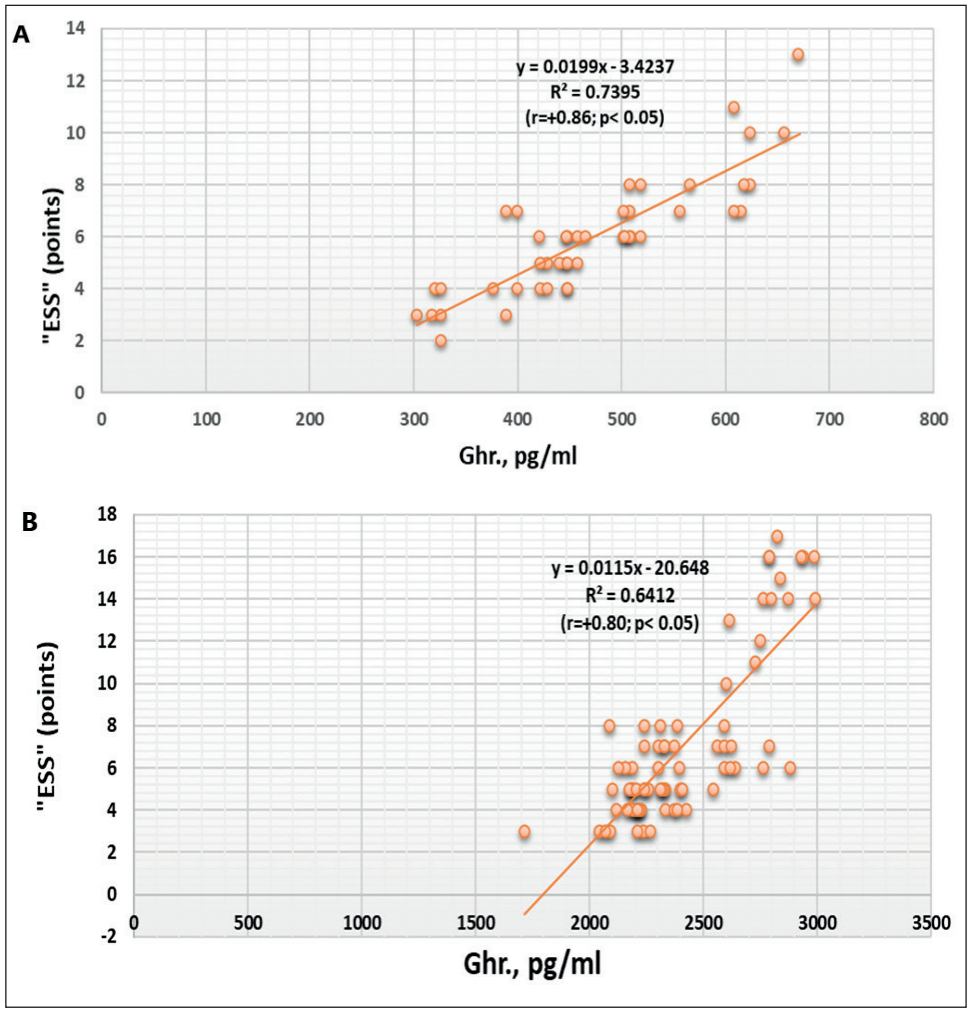

Fig. 5. Relation between EDS index on The Epworth Sleepiness Scale and ghrelin level in the patients with GERD (A) and in the patients with GERD associated with UCTD (B). Correlation field and regression line (with equation) describing it. ($r=+0.80$; $p<0.05$); R^2 is the determination coefficient.

A direct correlation between the severity of excessive daytime sleepiness and the level of ghrelin was also established: I Group – r = + 0.86; p<0.05; II Group – r = +0.80, p<0.05. The obtained results were comparable to the following scientific data. Circulating in the body, ghrelin may impact the circadian system as a potential feedback signal. Laermans J. and co-workers have found that ghrelin can regulate peripheral circadian rhythm fluctuations [24]. Since the circadian system of the body is highly-sensitive, the latter authors assume that its imbalance may be considered as the first manifestations of the disease. Motivala S. and co-author's study published in the Psychoneuroendocrinology journal experimentally found that chronic insomnia violated ghrelin circulation in the body contributing to its decrease at night and increase during the day. The same tendency was observed in the cases of the reduction of falling asleep time and decrease in total sleep time [15]. Hagen E. and co-authors found that there was significant inverse relationship between ghrelin and sleep duration (r=-0.18, p=0.04) [25]. A significant inverse association between sleep duration and ghrelin level was also confirmed in the works of Al-Disi D. [26] and Broussard J. [9].

CONCLUSIONS

Thus, the more severe were the manifestations of GERD and AET, the higher was the degree of excessive daytime sleepiness manifestation which had direct correlation with circulating ghrelin level. The possible role of ghrelin in the sleep regulation in the patients with GERD and UCTD combination was also indicated by the direct relation between the frequency of reflux with pH<4 and its duration over 5 minutes and ghrelin level in blood serum.

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Conflict of interest:
The Authors declare no conflict of interest.

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A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

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