ORIGINAL ARTICLE

CONTENTS

Peculiarities of changes in the level of vitamins B in patients with gastroesophageal reflux disease metabolic-associated fatty liver disease

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ABSTRACT

Aim: To determine the peculiarities of changes in the levels of B vitamins and their dependence on body mass index (BMI) in patients with GERD and metabolic dysfunction-associated fatty liver disease (MDAFLD).

Materials and Methods: 112 patients with GERD were examined. Patients were divided into two groups. Group 1 included 50 patients with GERD, and group 2 consisted of 62 patients with GERD in combination with MDAFLD. The level of homocysteine and B vitamins in the blood serum was determined.

Results: At patients group 1 were diagnosed the decreased levels of B6 and B12 in blood serum against a background of a slight increase in homocysteine levels in blood serum (up to 17.9±0.7 umol/l – $p<0.05$). In patients in group 2, a significant decrease in all B vitamins examined in the blood serum was found, accompanied by a significant increase in homocysteine levels (2.4 times – $p<0.01$).

Conclusions: In the vast majority of patients with GERD and MDAFLD, an increase in BMI was found, namely, overweight in 25.8% ($p < 0.01$) of patients and grade I obesity (in 37.1% of patients – $p < 0.001$). In patients with GERD and MDAFLD, a significant decrease in serum levels of B vitamins (B1, B3, B6, B9, B12) was found, which correlates negatively with overweight and obesity in these patients.

KEY WORDS: metabolic dysfunction-associated fatty liver disease (non-alcoholic fatty liver disease), gastroesophageal reflux disease, obesity, B vitamins, homocysteine

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common liver pathology worldwide, affecting 20-30% of the global population [1, 2]. Non-alcoholic fatty liver disease is characterised by the accumulation of macrovacuolar fat in the liver in individuals with no history of other aetiological factors, including excessive alcohol consumption. In the context of the obesity pandemic, according to experts, NAFLD affects more than 64 million people in the United States [3]. It has been established that NAFLD can not only progress to cirrhosis and even liver cancer, but is also closely associated with an increased risk of serious extrahepatic diseases, such as cardiovascular disease (CVD) and metabolic syndrome (MS). People with NAFLD are twice as likely to die from CVD than from liver disease. In light of the above information, a new definition has recently been proposed, called metabolic dysfunction associated with fatty liver disease (MDAFLD). To diagnose MDAFLD, it is no longer necessary to rule out other liver diseases, such as excessive alcohol consumption, viral hepatitis infection, and autoimmune liver diseases. Metabolic dysfunction is necessary for the correct verification of the diagnosis of MDAFLD [4, 5].

Gastroesophageal reflux disease (GERD) is a disease of the upper gastrointestinal tract characterised by reflux of stomach contents into the oesophagus and manifested by symptoms such as heartburn, belching and dysphagia. GERD is more commonly diagnosed in Western countries with high economic development, where its prevalence is estimated at 20%, compared to <5% in Asian countries [3]. The prevalence of GERD is about 13%, and unsatisfactory treatment results negatively affect the quality of life of such patients. Metabolic syndrome has been shown to be both an independent risk factor for GERD and a well-known risk factor for fatty liver disease [1].

There are no specific drugs for the treatment of NAFLD. Lifestyle modification remains the most effective long-term method of correction, and dietary recommendations are widely considered to be its basis [6, 7]. Given the significant impact of vitamins on the immune

system and their potential to influence NAFLD, it is advisable to investigate the role and level of vitamin intake in the diet of such patients. Some studies have examined the role of vitamins D, C, and E in the treatment of NAFLD [8, 9], but there is a lack of research on B vitamins and NAFLD, especially in the context of comorbidities with GERD.

AIM

To determine the peculiarities of changes in the levels of B vitamins and their dependence on body mass index (BMI) in patients with GERD and metabolic dysfunction-associated fatty liver disease (MDAFLD).

MATERIALS AND METHODS

At the clinical base of the Department of Procedure of Internal Diseases, 112 patients with GERD were examined. The examined patients with GERD for the period 2020 to 2025 were treated in the gastroenterological and endocrinological departments of the Municipal Non-Profit Enterprise «Andriy Novak Transcarpathian Regional Clinical Hospital» of the Transcarpathian Regional Council.

Patients were divided into two groups. Group 1 included 50 patients with GERD, and group 2 consisted of 62 patients with GERD in combination with MDAFLD. Among the examined patients of group 1, there were 25 (50.0 %) men, with an average age of 42.9±4.5 years; there were 25 (50.0 %) women, with an average age of 39.9±6.1 years. Among the examined patients of group 2, there were 39 (62.9 %) men, with an average age of 30.7±4.9 years; there were 23 (37.1 %) women, with an average age of 28.9±5.6 years. The control group included 30 healthy individuals (19 (56.7 %) men and 11 (43.3 %) women). The average age was 35.5±4.7 years.

The studies were conducted with patient approval (written authorization for relevant diagnostic procedures and therapeutic interventions was secured from all participants and control group members) while implementing comprehensive measures to maintain confidentiality of the collected data. The research methodology complied with the 1975 Helsinki Declaration on Human Rights and its 1983 revision, the Council of Europe Convention on Human Rights and Biomedicine, as well as Ukrainian legal requirements.

GERD diagnosis was established based on the unified clinical protocol criteria (Ministry of Health of Ukraine order No. 943 dated 31.10.2013) incorporating patient symptoms, endoscopic findings, and other relevant data. For diagnostic confirmation, study participants underwent fibroesophagogastroduodenoscopy (FEGDS) utilizing Pentax ERM-3300 video processor endoscopy equipment and Pentax E-2430, GIF-K20 flexible fiber endoscopes. Additionally, 24-hour pH monitoring was conducted following Prof. V.N. Chernobrovy's methodology. Endoscopic evaluation of esophageal damage severity employed the Los Angeles (LA) classification system (1998): Grade A – single (or multiple) mucosal defect measuring 5 mm or less, not extending between mucosal fold summits; Grade B – single (or multiple) mucosal defect exceeding 5 mm in length, not extending between mucosal fold summits; Grade C – single (or multiple) mucosal defect bridging two or more mucosal fold summits while affecting less than 75% of the circumference; Grade D – single (or multiple) mucosal defect encompassing at least 75% of the esophageal circumference.

MDAFLD diagnosis was established following the criteria outlined in the unified clinical protocol (Ministry of Health of Ukraine Order No. 826 dated 06.11.2014) and the EASL-EASD-EASO clinical recommendations for managing and treating such patients. The extent of hepatic impairment was assessed utilizing web-based calculators including NAFLD fibrosis score (NFS), Fibrosis 4 calculator (FIB-4), fibrotest, FibroIndex, Forns, APRI, along with hepatic elastometry findings.

All study participants underwent comprehensive evaluation using general clinical, anthropometric, instrumental, and laboratory techniques. To confirm the diagnosis, detailed patient complaints and medical history were thoroughly documented. Anthropometric assessment included measurements of height, weight, and waist circumference, with subsequent BMI calculation. Following WHO guidelines, participants were categorized based on BMI values: 16.0 or below indicated severe underweight; 16.0-18.5 represented underweight; 18.0-24.9 denoted normal weight; 25.0-29.9 signified overweight; 30.0-34.9 corresponded to first-degree obesity; 35.0-39.9 indicated second-degree obesity; 40.0 and above represented third-degree obesity.

The exclusion criteria were as follows: age under 18 years and over 70 years, liver damage due to viral (hepatitis B, C, D viruses), alcohol etiology; haemochromatosis; Wilson-Conovalov disease; oesophageal adenocarcinoma; stomach or duodenal ulcer; systemic autoimmune diseases; oncological diseases; pulmonary tuberculosis; psychiatric diseases that do not allow adequate assessment of the patient's health status and signing an informed consent for diagnosis and treatment; pregnancy and lactation; acute myocardial infarction, stroke (in the history of up to 6 months).

Serum homocysteine levels were determined in all study participants using the Cobas 8000 testing

platform (Roche Diagnostics), alongside B vitamin assessments: vitamin B1 (thiamine), vitamin B3 (niacin) and vitamin B6 (pyridoxine) were analyzed through high-performance liquid chromatography utilizing test kits (Recipe Complete Kit, Germany); vitamin B9 (folic acid) was measured via immunochemical methodology with electrochemiluminescent detection employing the Roche Diagnostics testing platform (Switzerland), while vitamin B12 (cyanocobalamin) was quantified through immunochemical chemiluminescent detection using the Abbott Diagnostics testing platform (USA).

The analysis and processing of the results of the examined patients were performed with the help of the computer program STATISTICA 10.0 (StatSoft Inc, USA) using parametric and non-parametric methods of evaluation of the results.

RESULTS

Changes in body mass index in the examined patients were assessed – Table 1.

Analysis of the anthropometric data obtained indicates a predominance of individuals with grade I obesity among patients in group 2 (in 37.1% of those examined – $p < 0.001$), while in group 1, patients with excess body weight were more common (44.0% of patients – $p < 0.01$). It should be noted that no patients with grade III obesity were diagnosed in group 1, while 6.4% of patients in group 2 were diagnosed with grade III obesity. Only in group 1 were patients with body mass deficit and significant body weight deficiency. When analysing the anamnestic data, it was during the stage of weight loss (among patients in group 1) that the onset of clinical manifestations of GERD was diagnosed.

The levels of B vitamins and homocysteine in the blood serum of the examined patients were assessed – Table 2.

At patients group 1 with GERD were diagnosed the decreased levels of B6 and B12 in blood serum against a background of a slight increase in homocysteine levels in blood serum (up to 17.9±0.7 umol/l, compared to 11.8±0.9 umol/l in the control group – $p<0.05$). The levels of vitamins B1, B3 and B9 in the blood serum of patients in group 1 also tended to decrease compared to those in the control group, but did not exceed the reference values.

In patients in group 2 (GERD combined with MDAFLD), a significant decrease in all B vitamins examined in the blood serum was found, accompanied by a significant increase in homocysteine levels (2.4 times – $p<0.01$).

A statistical analysis was performed to assess the relationship between vitamin levels and BMI at the examined patients – Table 3, 4.

Correlation analysis indicates a relationship between changes in BMI and levels of B vitamins. At the same time, a decrease in B vitamin levels correlates negatively with excess body weight and obesity of varying severity, predominantly in patients in group 2. Homocysteine levels are directly dependent on BMI, which corresponds to overweight and grade I obesity (in patients in group 1), as well as on an increase in BMI (overweight and grades I-II obesity) in patients in group 2.

Thus, our data indicate a relationship between increased body weight and decreased levels of B vitamins in blood serum in GERD combined with NAFLD.

DISCUSSION

A lot of cohort and observational studies have displayed that people suffering from NAFLD had a higher susceptibility to GERD, and one clinical trial indicated that the risk of reflux esophagitis would increase 16% among people with NAFLD (OR 1.16 95% CI 1.13–1.20) as compared to those without NAFLD9,10,11,12. In addition, a meta-analysis showed that the incidence of NAFLD was higher in people with GERD compared with healthy individuals, with an amalgamated OR of 2.07 (OR 2.07 95% CI, 1.54–2.79) [10].

Catanzaro R. and colleagues (2014) show that the prevalence of typical GERD symptoms is higher in patients with NAFLD. GERD was associated with higher BMI and metabolic status, but not with age and type 2 diabetes. According to these results, MetS can be considered as a common background, but it cannot fully explain this correlation. The authors suggest that NAFLD is an independent risk factor for the development of GERD symptoms [11].

Diet plays a significant role in choosing treatment tactics for patients with NAFLD. However, clinical studies on the consumption of foods rich in B vitamins, particularly in cases of concomitant GERD, are insufficiently studied.

The following compounds belong to the water-soluble B vitamins: vitamin B1 (thiamine), vitamin B2 (riboflavin), vitamin B3 (niacin), vitamin B5 (pantothenic acid), vitamin B6 (pyridoxine), vitamin B7 (biotin), vitamin B9 (folic acid), vitamin B12 (cyanocobalamin). However, only the levels of individual B vitamins have been studied in patients with NAFLD. Vitamins B1 and B2 and their active coenzymes (thiamine pyrophosphate and flavin adenine dinucleotide) play an important role in the catabolism of carbohydrates and fatty acids. The study found that premenopausal women, compared to postmenopausal women, are unable to synthesise choline (vitamin B4) through the endogenous pathway catalysed by phosphatidyl ethanolamine N-methyltransferase due to the absence or reduction of oestrogen levels [12]. The authors associate more severe fibrosis in

Table 1. Distribution of examined patients depending on BMI

Indicator	Examined patients	
	Group 1 (n=50) Absolute number / %	Group 2 (n=62) Absolute number / %
Significant body weight deficiency (BMI: less than 16.0)	1 / 2.0 %	_
Body mass deficit (BMI: 16.0–18.5)	5 / 10.0 %	_
Normal weight (BMI: 18.5 – 24.9)	16 / 32.0 % *	7 / 11.3 %
Overweight (BMI: 25.0 – 29.9)	22 / 44.0 % *	16 / 25.8 %
Obesity, grade I (BMI: 30.0 – 34.9)	4 / 8.0 %	23 / 37.1 % **
Obesity, grade II (BMI: 35.0 – 39.9)	2 / 4.0 %	12 / 19.4 %
Obesity, grade III (BMI: more than 40.0)	_	4 / 6.4 %

Note: statistically significant difference between indicators in patients in groups 1 and 2: * – $p < 0.01$; ** - $p < 0.001$
Source: compiled by the authors of this study

Table 2. Levels of B vitamins and homocysteine in the blood serum of the examined patients

Indicator	Reference values	Examined subjectes		
		Control group (n=20)	Group 1 (n=50)	Group 2 (n=62)
Vitamin B1, nmol/l	40.0-80.0	65.3±1.7	46.5±0.7+	31.8±0.6++*
Vitamin B3, umol/l	4.7-8.34	7.2±0.8	5.5±0.3+	2.9±0.4++*
Vitamin B6, ug/ml	14.6-72.8	58.9±2.4	13.9±1.1++	9.3±0.3+++*
Vitamin B9, ng/ml	3.0-17.0	12.6±0.6	4.8±0.2++	2.2±0.3+++**
Vitamin B12, pg/ml	197.0-771.0	621.5±5.7	188.5±3.2+++	143.7±2.5+++*
Homocysteine, umol/l	5.0-15.0	11.8±0.9	17.9±0.7+	28.3±0.4++*

Note: differences between the control group and the examined patients are significant: + – $p<0.05$; ++ – $p<0.01$; +++ – $p<0.001$; the difference between the indicators in patients of groups 1 and 2 is statistically significant: * – $p<0.05$; ** – $p<0.01$
Source: compiled by the authors of this study

Table 3. Comparison of BMI with levels of B vitamins and homocysteine in examined patients of group 1

Indicator	Examined patients of group 1		
	Normal weight	Overweight	Obesity, grade I
Vitamin B1	_	_	r= -0,70; p<0,05
Vitamin B3	_	_	r= -0,76; p<0,05
Vitamin B6	r= 0,66; p<0,05	r= -0,68; p<0,05	r= -0,80; p<0,01
Vitamin B9	_	r= -0,70; p<0,05	r= -0,74; p<0,05
Vitamin B12	r= 0,70; p<0,05	r= -0,74; p<0,05	r= -0,78; p<0,01
Homocysteine	r= -0,78; p<0,01	r= 0,70; p<0,05	r= 0,76; p<0,01

Source: compiled by the authors of this study

postmenopausal women with low choline intake. Vitamin B3 (niacin) is a precursor of the coenzyme nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate and is considered a possible option for the treatment of NAFLD. However, the relationship between vitamin B12, vitamin B9 and NAFLD is currently under debate due to the lack of clinical evidence of their possible positive effects in NAFLD [13-15].

A study conducted by Dr. Tripathi and his colleagues investigated the effects of vitamin B12 and folates in dietary models of mice and Cbs knockout mice, as well as in 36 patients and primates. After adding folates and vitamin

Table 4. Comparison of BMI with levels of B vitamins and homocysteine in examined patients of group 2

Indicator	Examined patients of group 2			
	Normal weight	Overweight	Obesity, grade I	Obesity, grade II
Vitamin B1	_	r= -0,54; p<0,05	r= -0,74; p<0,05	r= -0,78; p<0,01
Vitamin B3	_	r= -0,68; p<0,05	r= -0,82; p<0,01	r= -0,86; p<0,01
Vitamin B6	r= 0,74; p<0,05	r= -0,72; p<0,05	r= -0,86; p<0,01	r= -0,84; p<0,01
Vitamin B9	r= 0,68; p<0,05	r= -0,68; p<0,05	r= -0,90; p<0,01	r= -0,82; p<0,01
Vitamin B12	r= 0,76; p<0,01	r= -0,70; p<0,05	r= -0,92; p<0,01	r= -0,86; p<0,01
Homocysteine	r= -0,80; p<0,01	r= 0,68; p<0,05	r= 0,80; p<0,01	r= 0,86; p<0,01

Source: compiled by the authors of this study

B12 to drinking water from 0 to 16 weeks, the authors found histological improvement in liver cell infiltration by inflammatory cells and fibrosis in mice (except for steatosis). These data open up new possibilities for the use of dietary supplements containing vitamin B12 and folates for the prevention or treatment of NAFLD [16].

The role of B vitamins in patients with GERD is also not unambiguous. Sharp L and al. (2013) indicate that riboflavin intake is inversely related to reflux oesophagitis, and vitamin B-12 intake is positively related to esophageal adenocarcinoma (EAC). According to the authors, folate and other dietary factors containing methyl groups are involved in the etiology of EAC and its precursors [17].

On the contrary, another study investigated the role of a dietary supplement containing melatonin, l-tryptophan, vitamin B6, B9, B12, methionine and betaine on clinical symptoms of GERD. A blinded, randomised study was conducted in which 176 patients were treated with the above supplement (group A) and 175 were treated with omeprazole at a dose of 20 mg (group B). All subjects in group A (100%) reported complete regression of symptoms after 40 days of the proposed treatment. Only 115 patients (65.7%) taking omeprazole reported regression of symptoms over the same period ($p < 0.05$). The authors concluded that the drug they tested promotes regression of GERD symptoms without significant side effects [18].

Our data also indicate a marked decrease in the levels of all B vitamins (B1, B3, B6, B9, B12) in the blood serum of patients with GERD combined with MDAFLD. A correlation was established between BMI and a decrease in the levels of the corresponding vitamins, which requires correction. Further research is needed to clarify the relationship between B vitamins and obesity of varying severity in GERD combined with MDAFLD.

CONCLUSIONS

1. In the vast majority of patients with GERD and MDAFLD, an increase in BMI was found, namely, overweight in 25.8% ($p < 0.01$) of patients and grade I obesity (in 37.1% of patients – $p < 0.001$).
2. In patients with GERD and MDAFLD, a significant decrease in serum levels of B vitamins (B1, B3, B6, B9, B12) was found, which correlates negatively with overweight and obesity in these patients.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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