Nutritional Biomarkers in Patients with Obesity - the Relation Between Helicobacter Pylori Infection and Micronutrients

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Obesity has experienced an epidemic evolution over the last years, affecting all age groups. Helicobacter pylori (H. pylori) infection affects over half the world's population and the association with obesity can worsen the nutritional status of these patients. The aim of this study was to assess the nutritional biomarkers (minerals and vitamins) in obese patients in relation to H. pylori infection. Included in the study were 93 obese patients, 47 of which (50.5%) were infected with H. pylori (H. pylori present) confirmed by histology. Several clinical parameters (weight, body mass index, waist circumference and blood pressure) and nutritional biomarkers represented by micronutrients (minerals and vitamins) were analyzed. Our results showed that the levels of folic acid, total calcium and ionized calcium were higher in patients without H. pylori infection and the levels of vitamin B12, vitamin D, magnesium, serum iron and ferritin were higher in patients with H. pylori infection, but the differences were not statistically significant ($p>0.05$). In conclusion, in our obese patients the H. pylori infection does not influence the nutritional biomarkers represented by micronutrients (vitamins and minerals) in the evaluation of nutritional status.

Keywords: nutritional biomarkers, micronutrients, obesity, Helicobacter pylori

Helicobacter pylori (H. pylori) is a gram negative bacterium widespread globally, the *H. pylori* infection prevalence being estimated at over 50% of the world population. It has a predominant distribution in developing countries, and the poor socioeconomic status is an important risk factor [1]. It is a pathogen that induces an inflammatory response in the gastric mucosa, being associated with lesions of atrophic and non-atrophic gastritis, peptic ulcer disease; a strong association with lymphoma and gastric adenocarcinoma has also been demonstrated [2]. The alteration of gastric mucosa in the context of *H. pylori* infection will in turn influence not only the absorption of nutrients, but also the level of gastrointestinal peptides (involved in appetite regulation and energy metabolism). Defining the role of *H. pylori* infection on nutritional status was a priority for numerous research groups, and the results were often contradictory. Some studies have demonstrated an association between *H. pylori* infection and iron deficiency anemia, which is even more important in children compared to adults [3-5]. Others showed that *H. pylori* infection can cause deficiency of certain vitamins (fat-soluble or water-soluble) and minerals. Published researches showed the association between *H. pylori* infection and B12 hypovitaminosis [6, 7], β-caroten [8] and α-tocoferol deficiency [9] in the gastric juice, with C hypovitaminosis [10] and folic acid deficiency [9]. As to the relationship between *H. pylori* infection and serum levels of certain minerals, the data are much scarcer and controversial, and often related to zinc [4, 11] or selenium [12].

The relationship between *H. pylori* infection and weight status is also controversial. However, numerous studies have demonstrated weight gain and an increased incidence of obesity after the eradication therapy of *H. pylori* infection [13], due to increased plasma ghrelin levels, among others [14]. Overweight people often associate numerous nutritional deficiencies, regardless of *H. pylori* infection, partly due to restrictive and repeated diets in an attempt to get a proper weight control.

The aim of our study was to evaluate some biochemical parameters for nutritional assessment (micronutrients - vitamins and minerals) in a group of obese patients, and to evaluate the influence of *H. pylori* infection on their nutritional status.

Experimental part

Material and method

Cross-sectional observational study which included 93 obese patients evaluated in terms of metabolic and nutritional parameters by a multidisciplinary team in the interval 2014-2016. The inclusion criteria were: age over 18, no known digestive and kidney diseases, no treatment with nutritional supplements in the past year, no malabsorption-associated disorders. The study protocol was previously approved by the Ethics Committee and the

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patients included in the study read and signed an informed consent form before any study procedures.

Anthropometric parameters were assessed according to the recommendations of the World Health Organization [15] and for each subject the following were recorded: current weight, waist circumference and body mass index calculated based on weight and height, according to the formula BMI = weight (kg)/height (m^2).

Biological samples (venous blood) were collected and serum fasting levels of the studied micronutrients - vitamins (vitamin B12, vitamin D, folic acid) and minerals (total calcium, ionic calcium, serum iron, ferritin, magnesium) were measured in the same laboratory.

The method for measuring calcium levels was calcium dye complexes, the procedure based on arsenazo-III dye reacting with calcium in an acid solution to form a blue-purple complex. The color developed is measured at 660 nm and is proportional to the calcium concentration in the sample (kit Abbot Laboratories REF 3L79). For measuring iron levels the MULTIGENT Iron assay was used, which is a method intended for the direct colorimetric determination of iron without deproteinization in human serum or plasma on the ARCHITECT cSystem. At a *p*H of 4.8, iron is released from transferrin to which it is bound, and then quantitatively reduced to a ferrous state. The iron forms with Ferene-S (3-(2-pyridyl)-5,6-bis-[2-(5-furylsulfonic acid)]-1,2,4-triazine) a stable colored complex the intensity of the developed color being proportional to the amount of iron in the sample (kit Abbot Laboratories REF 6K95-30 and 6K95-41 for use with Architect). For measuring ferritin levels Quantia Ferritin reagent was used, which is a suspension of polystyrene latex particles of uniform size coated with rabbit IgG anti-human ferritin. When a sample containing ferritin is mixed with the reagent, a clear agglutination occurs, which can be measured by turbidimetry (kit Abbot Laboratories REF 6K41-01 for use with Architect). For measuring magnesium levels, an enzymatic assay kit was used. Magnesium present in the sample is a cofactor in an enzymatic reaction with isocitrate dehydrogenase. The rate of increase in absorbance at 340 nm, due to the formation of NADPH, is directly proportional to the magnesium concentration (kit Abbot Laboratories REF 3P68 305531/R02). For measuring vitamin D, B12 and folic acid levels, the electrochemiluminescence binding assay intended for use on cobas e 411 immunoassay analyzers was used. Vitamin D total assay employs a vitamin D binding protein as capture protein to bind vitamin D_3 (25-OH) and vitamin D_2 (25-OH). Vitamin B12 assay employs a competitive test principle using intrinsic factor specific for vitamin B12. Vitamin B12 in the sample competes with the added vitamin B12 labeled with biotin for the binding sites on the ruthenium-labelled intrinsic factor complex. The folate assay employs a competitive test principle using natural folate binding protein specific for folate. Folate in the sample competes with the added folate (labeled with biotin) for the binding sites on folate binding protein (labeled with ruthenium complex).

All patients included in the study underwent upper gastrointestinal endoscopy, performed by the same experienced gastroenterologist, who took gastric mucosa biopsies, which were immediately sent to Pathology Department for morphological evaluation. The macroscopic appearances seen during the endoscopy were divided into four categories: normal, congestion, gastritis, and other lesions (granular aspect, hypertrophic folds, and biliary reflux). Histological examination of the gastric biopsy provided two types of information regarding gastritis: classification and grading of inflammation (which resulted into three categories: normal, superficial chronic gastritis and deep chronic gastritis), and assessment of *H. pylori* infection [16].

Data were analyzed using Microsoft Office Excel and SPSS version 17.0. Numerical data were expressed as means and standard deviation (SD), minimum and maximum and significant differences between numerical data were found using *t* student test. For different than normal distributions we applied nonparametric tests, for distributions close to normal we applied the t test, with a significance value of <0.05.

The ethics committee of the university granted approval for the study and all the patients gave their consent to participate.

Results and discussions

The study included 93 obese patients, 47 of which (50.5%) had *H. pylori* infection diagnosed by the pathologic examination of biopsy piece obtained by the endoscopy. The clinical characteristics of the two study subgroups (*H. pylori* + versus *H. pylori* -) are shown in Table 1. The results showed that patients with *H. pylori* infection had higher values for weight and waist circumference, but the differences were not statistically significant between the two subgroups ($p>0.05$).

The literature data on the prevalence of *H. pylori* infection in patients with morbid obesity are highly variable; a prevalence ranging from 8.7% in a German cohort [17] to 85.5% in a Saudi Arabian cohort [18] has been reported, and these major differences can be explained in part by the use of different diagnostic methods and different research methods. Many authors assessed the association between weight status and the prevalence of *H. pylori* infection, and the results were controversial. Thus, a 2014 analysis of 50 studies that included 99,000 subjects showed that the prevalence of overweight and obesity was

Table 1

CLINICAL CHARACTERISTICS OF THE TWO STUDY SUBGROUPS

		Mean	95% CI	Median	Std. dev	Min	Max	p
Weight (kg)	*H. pylori* absent	121.750	115.617-127.883	117	20.654	93	180	
	H. pylori present	126.415	119.785-133.045	121	22.580	90	175	0.312
BMI (kg/m^2)	*H. pylori* absent	44.27	42.219-46.334	43.013	6.927	33.83	66.51	
	H. pylori present	44.04	42.264-45.823	43.306	5.992	34.53	60.60	0.978
WC (cm)	*H. pylori* absent	125.102	120.315-129.890	127	15.747	88	156	
	H. pylori present	128.913	124.413-133.413	130	15.153	102	158	0.245
SBP (mmHg)	*H. pylori* absent	132.591	127.697-137.485	130	16.097	100	160	
	H. pylori present	135.087	129.411-140.763	130	19.112	110	180	0.733
DBP (mmHg)	*H. pylori* absent	83.864	80.624-87.103	81	10.654	50	108	
	H. pylori present	80.891	77.896-83.887	80	10.086	50	100	0.135

Table 2

THE LEVEL OF MICRONUTRIENTS (VITAMINS AND MINERALS) IN THE STUDY GROUPS OF OBESE PATIENTS

		Mean	95% CI	Median	Std. dev.	Min	Max	p
Vitamin B12 (pg/mL)	*H. pylori* absent *H. pylori* present	328.51 333.78	289.33-367.69 297.45-370.11	291 308	124.12 115.11	150 150	655 600	0.603
Vitamin D (ng/mL)	*H. pylori* absent *H. pylori* present	14.70 17.25	12.29-17.10 13.93-20.58	12.87 14.70	7.72 10.92	3 3.3	36.44 57.08	0.432
Folic acid (ng/mL)	*H. pylori* absent *H. pylori* present	6.34 5.96	5.46-7.23 4.80-7.11	6.02 5.38	2.53 3.51	1.8 2.38	12.2 24	0.164
Total Ca (mg/dL)	*H. pylori* absent *H. pylori* present	9.5 9.39	9.36-9.63 9.26-9.53	9.41 9.41	0.44 0.46	8.73 8.2	10.50 10.67	0.291
Ionic Ca (mg/dL)	*H. pylori* absent *H. pylori* present	4.38 4.27	4.21-4.55 4.11-4.42	4.21 4.18	0.56 0.48	2.3 2.89	5.4 5.12	0.257
Magnesium (mg/dL)	*H. pylori* absent *H. pylori* present	2.06 2.09	1.99-2.12 2.04-2.15	2.07 2.11	0.2 0.175	1.22 1.66	2.49 2.43	0.357
Serum iron (microg/dL)	*H. pylori* absent *H. pylori* present	78.43 86.17	70.06-86.79 73.99-98.357	71.5 81	27.51 40.53	31 32	145 221	0.585
Ferritin (ng/mL)	*H. pylori* absent *H. pylori* present	83.86 123.82	61.03-106.69 68.90-178.74	64 69	60.01 149.72	7 18	200 767	0.336

inversely correlated with the prevalence of *H. pylori* infection, statistically significant ($r = -0.292$, $p < 0.05$ and $r = -0.43$, $p < 0.01$), respectively [19]. Another study published in 2009 showed a higher prevalence of *H. pylori* infection in obese patients compared to normal weight patients [20], while a national cross-sectional study conducted on a significant and representative sample of the US population, involving over 7000 subjects, did not show any relationship between *H. pylori* infection and weight status assessed by anthropometric parameters (weight and BMI), even after adjusting for demographic variables or for those related to lifestyle [21].

H. pylori infection causes changes in the gastric mucosa that will influence food intake, digestion and absorption of ingested food, as such being associated with secondary nutritional deficiencies. The risk of a nutritional deficiency is especially important in obese patients, on frequently repeated previous restrictive diets, that ultimately lead to malnutrition. Table 2 presents the results obtained by the assessment of nutritional parameters - vitamins and minerals - in our group of patients with obesity, results presented comparatively according to the presence or absence of histologically diagnosed *H. pylori* infection.

The results showed that plasma folic acid, ionic calcium and total calcium levels were higher in the obese patients without *H. pylori* infection, but the differences were not statistically significant ($p > 0.05$). In contrast, plasma levels of vitamin B12, vitamin D, magnesium, serum iron and ferritin were higher in patients with *H. pylori* infection; these differences were also not statistically significant ($p > 0.05$).

The literature data regarding the influence of *H. pylori* infection on nutritional status are controversial. A study published in 2015 on a sample of 140 patients (over 60% obese) did not find statistically significant differences in terms of nutritional status and dietary intake between patients with or without *H. pylori* infection [22]. Epidemiological studies have shown a relationship between *H. pylori* infection and iron deficiency anemia. A Danish study conducted on a sample of over 2,700 subjects showed a reduction in serum ferritin levels in patients with high *H. pylori* antibody *titers* [23] and several case reports have shown unexplained iron deficiency anemia correction after therapy for eradication of *H. pylori* infection [24, 25]. However, there is evidence that *H. pylori* infection does not alter plasma levels of iron and hemoglobin, and that the presence of *H. pylori* infection does not influence the long-term effectiveness of food fortification with iron and vitamin B12 in children with malnutrition [26].

We found much less data in the literature regarding serum levels of other minerals in *H. pylori* infection, but many authors suggest its involvement in the development of zinc, copper or calcium deficiency [24, 25].

H. pylori infection seems to also be involved in the development of vitamin deficiency, such as vitamin A, vitamin C, vitamin B12 and folic acid [27]. With regard to the impact of *H. pylori* infection on vitamin B12 and folic acid levels the literature data are also controversial. Thus, a study published in 2012 showed that there is no association between plasma vitamin B12 and folic acid levels and *H. pylori* infection [28], while another study on an adult population showed a statistically significant relationship between *H. pylori* infection and serum vitamin B12 levels, regardless of the degree of gastric atrophy [4]. Much less data are found in the literature regarding the relationship between vitamin D levels and *H. pylori*, infection, most authors recommending further studies with larger sample sizes and more diverse populations.

Our study aimed to assess the level of micronutrients used in nutritional assessment of obese patients according to the presence of *H. pylori* infection. To the best of our knowledge, it is the first study of its kind conducted in a population with these characteristics in Romania. A recently published study attempted to evaluate the impact of energy-restricted diets on vitamin and mineral intake in a population of overweight patients [29], but without providing data related to the infection with *H. pylori*. During the last year more studies have been published by various authors, but the nutritional assessment was conducted in the context of severe diseases with a possible impact on food intake and nutritional status, such as in patients with chronic kidney disease [30], HIV [31] or oral cancers [32], and without bringing into discussion the evaluation of *H. pylori* infection. Our study has its limitations. One of them is that we did not present data related to individual food intake, which could influence the results on plasma micronutrient levels. Another limitation of this study is the lack of data on the socioeconomic status of the enrolled

patients, which could contribute to differences in nutritional parameters.

Conclusions

Our study showed that obese patients with *H. pylori* infection have a higher weight, abdominal circumference and systolic blood pressure compared to those without *H. pylori* infection ($p>0.05$). Regarding biochemical parameters for nutritional assessment, our results showed that the folic acid, total calcium and ionic calcium levels are higher in those without *H. pylori* infection, and the levels of vitamin B12, vitamin D, magnesium, serum iron and ferritin are higher in those infected with *H. pylori*, but the differences were not statistically significant ($p>0.05$). We can therefore say that in our group of obese patients *H. pylori* infection does not influence the micronutrient levels in nutritional assessment.

Acknowledgements: This research was financed by the Grigore T. Popa University of Medicine and Pharmacy by contract no. 30887/ 30.12.2014.

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Manuscript received: 16.05.2016