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Does *Helicobacter pylori* eradication effect body composition, dietary intake, serum leptin and ghrelin levels of infected patients?

[*Helicobacter pylori* eradikasyonu enfekte hastaların vücut bileşimini, diyetle alımını, serum leptin ve ghrelin düzeylerini etkiler mi?]*

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ABSTRACT

Objective: Take in to account the relationship between obesity and many diseases and contradictory published results considering the effects of *H. pylori* infection on leptin and ghrelin levels, we decided to determine the effect of *H. pylori* eradication on body composition, dietary intake, leptin and ghrelin levels of infected patients.

Methods: This study included 100 patients. After endoscopy, active infection with *H. pylori* was determined by rapid urease test and histopathology evaluation. Eradication was confirmed by the urea breath test at 3 months. The body weight, body composition and dietary intake of patients were assessed by Seca scale, Maltron Bioscan 916 and 24-hour recall food questionnaire respectively before and after eradication. Serum leptin and ghrelin were determined by enzyme linked immunosorbent assay (ELISA) kits.

Results: The mean body weight, fat mass and body cell mass of patients increased after eradication but only the changes of body weight was statistically significant (P=0.01). The mean free fat mass and percentage of free fat mass decreased significantly at the end of study (P<0.05). Eradication has no significant effect on dietary intake, serum leptin and ghrelin levels.

Conclusion: According to our findings, eradication of *H. pylori* lead to a statistically significant increase of body weight and fat mass in patients while dietary intake, serum leptin and ghrelin levels of subjects did not change after treatment. It seems that enhanced incidence of gastro-esophageal reflux disease after *H. pylori* eradication may be due to increased body weight of these patients. Therefore dietary consulting can be helpful in *H. pylori* infected patients for preventing of weight gain after eradication.

Key Words: *Helicobacter pylori* eradication, body composition, serum ghrelin , serum leptin, dietary intake

Conflict of Interest: The authors declare no conflict of interest.

ÖZET

Amaç: Birçok hastalığın obesite ile olan ilişkisi gözönünde bulundurulduğunda, *H. pylori* enfeksiyonunun leptin ve ghrelin düzeyleri üzerine olan etkilerini dikkate alan, çelişkili sonuçlar içeren makaleler bulunmaktadır. Bizde çalışmamızda *H. pylori* ile enfekte hastalarda *H. pylori* eradikasyonunun vücut bileşimine, diyetle alıma, leptin ve ghrelin düzeyleri üzerine olan etkisini belirlemeyi kararlaştırdık.

Gereç ve Yöntemler: Bu çalışmaya 100 hasta dahil edildi**.** Endoskopi sonrasında, aktif *H. pylori* enfeksiyonu varlığı hızlı üreaz testi ve histopatolojik değerlendirme ile belirlendi. Eradikasyon ise 3. ayda üre nefes testi ile doğrulandı. Hastaların vücut ağırlığı, bileşimi ve diyetle alımı Seca scale, Maltron Bioscan 916 ve 24 saatlik yiyecek sorgu listesi ile sırasıyla eradikasyon öncesi ve sonrasında değerlendirildi. Serum leptin ve ghrelin düzeyleri enzim ilintili immun test (ELİSA) ile değerlendirildi.

Bulgular: Hastaların eradikasyon sonrasında ortalama vücut ağırlığı, yağ kütlesi ve vücut hücre kütlesi artmış olmasına rağmen yalnızca vücut ağırlığı istatistiksel olarak anlamlı idi (P=0.01). Çalışma sonunda ortalama serbest yağ kütlesi ve serbest yağ kütle yüzdesi belirgin olarak azaldı (P<0.05). Eradikasyonun yiyecek alımı, serum leptin ve ghrelin düzeylerine anlamlı etkisi yoktu.

Sonuç: Bulgularımıza göre hastalarda *H. pylori* eradikasyonu vücut ağırlığında ve yağ kütlesinde belirgin artışa neden olurken, tedavi sonrasında yiyecek alımı, serum leptin ve ghrelin düzeylerinde değişiklik görülmedi. Bu da gösteriyor ki, *H. pylori* eradikasyonu sonrasında gelişen gastro-özefagial reflü hastaların artmış vücut ağırlığından kaynaklanmaktadır. *H. pylori* ile enfekte hastalarda eradikasyon sonrasında oluşabilecek kilo alımını önlemek için diyet ile ilgili destek almak faydalı olacaktır.

Anahtar Kelimeler: *Helicobakter pylori* eradikasyonu, vücut bileşimi, serum ghrelin, serum leptin, diyetle alım

Çıkar Çatışması: Yazarlar herhangi bir çıkar çatışması bildirmemiştir.

Introduction

Helicobacter pylori is a gram negative, spiral shaped, micro-aerophilic bacillus which infects about half of the world's population [1,2]. *H. pylori* infection is contributed to the pathogenesis of gastritis, gastric and duodenal ulcer, gastric carcinoma, and mucosa associated lymphoid tissue lymphoma [3-5]. Moreover, *H. pylori* infection may be responsible for dyspeptic symptoms and decreased appetite [6,7]. It has been reported that successful eradication of *H. pylori* may be related with improved appetite and weight gain [8,9]. Gastritis due to *H. pylori* might lead to a reduction in BMI and food intake by some possible mechanisms. It has been postulated that *H. pylori* gastritis may damage the synthesis and secretion of leptin and ghrelin which are derived from stomach. These hormones affect appetite and adiposity [10]. Leptin is the peptide which is produced by adipocytes and by chief and endocrine P cells in the gastric epithelium [11,12]. Many factors especially body fat stores influence serum leptin levels and this hormone regulates body weight by suppressing food intake and increasing energy metabolism [12-14]. In addition, leptin help to maintain body weight by regulating intestinal nutrient absorption, delaying gastric emptying, sending signals short-term satiety by vagal afferent nerves, and is associated with Barrett's esophagus and increased gastric levels of leptin occur in *H. pylori* positive patients [15,16]. Ghrelin is a peptide hormone mainly produced by the X/A cells in the gastric oxyntic mucosa [17]. Ghrelin may lead to weight gain by stimulating food intake and decreasing energy expenditure [18]. Serum ghrelin levels are inversely related to adipose tissue mass and may regulate energy homeostasis when nutrients are insufficient [19]. However, conflicting results have been published regarding the effect of *H. pylori* infection on leptin and ghrelin levels [20,21]. Despite the findings of *H. pylori* eradication studies, by which mechanisms *H. pylori* infection could cause a reduction in BMI and food intake, is not well elucidated.

Increased body mass index (BMI) increases the risk of gastro-esophageal reflux symptoms, cancer, hypertension, diabetes, etc. Take in to account the relationship between obesity and many diseases and contradictory published results considering the effects of *H. pylori* infection on leptin and ghrelin levels, therefore, we decided to determine the effect of *H. pylori* eradication on body composition, dietary intake and leptin and ghrelin levels of infected patients.

Materials and Methods

This study was carried out in a group of patients from the Gastroentrological Clinic of the Emam Reza Hospital in Tabriz University of Medical Sciences who had been referred for endoscopic examination of the upper digestive tract. This study included 100 patients (45 men and 55 women; mean age 39.7±1.4 years).The protocol was approved by Ethics Committee of Tabriz University of Medical Sciences. Written Informed consent was obtained from all participants.Exclusion criteria were: age <20 year, pregnancy, diabetes mellitus, cachectic state including cancer, systemic infection, thyroid and liver and renal diseases, use of medications effective against *H. pylori* during the preceding 6 months, alcoholic abuse, drug addiction, and chronic corticosteroid or nonsteroidal anti-inflammatory drug use. None had undergone gastrointestinal surgery. To eliminate seasonal confounding factors, all patients were recruited during the summer.

Endoscopy was performed between 9:00 a.m. and 12:00 a.m. on all patients after overnight fasting and multiple biopsies were collected from the antrum and corpus. Active infection with *H. pylori* was determined by rapid urease test and histopathology evaluation. Infected patients were given a quadruple therapy (omeprazole 20 mg twice a day, colloidal bismuth subcitrate qid, amoxicillin 500 mg bid, clarithromycine 500 mg bid for two week) for eradication. After 2 weeks of eradication treatment, PPI (standard dose) was prescribed for 30 days as a sequential therapy**.** Eradication was confirmed by using the 13C-urea breath test 3 months after the end of therapy. According to the guidelines UBT should perform at least four weeks after completing treatment [22].

Height was measured using a mounted tape with the subjects' arms hanging freely by their sides and was recorded to the nearest 0.5 cm. After ensuring that subjects were barefoot and wore light clothing, their weight was recorded to the nearest 0.1 kg with a Seca scale at the onset and the end of the study. Body composition of patients (total body water, free fat mass and body fat) and resting metabolic rate (RMR) were determined by means of body impedance analysis (BIA; Maltron Bioscan 916, England; http://www.maltronint.com/products/bioscan916.php) before and after eradication. BioScan is a simple, practical, non-invasive, non-intrusive and a highly accurate portable diagnostic monitoring system for assessing body composition, nutritional and fluid monitoring in both healthy and clinically ill patients. It is calibrated for all age groups including different nationality. BioScan uses scientific patented method of measuring Electrical Impedance. A total of four electrodes were used (tetrapolar). 2 electrodes were applied to the hand and two to the foot. The electrodes should not be connected to an amputated or fistulated hand. It combines the power of modern electronics and proprietary digital signal processing to compute physical modelling of biological tissues. BioScan allows the estimation of many parameters, including total body water (TBW), intercellular water (ICW), extracellular water (ECW), fat free mass (FFM), fat mass (FM), body cell mass (BCM) and dry weight. Therefore, on the basis of the advantages of Bioscan in determining body composition, we decided to assess body composition of infected patients before and after eradication.

Dietary intake was assessed by using a 24-hour recall food questionnaire (two weekdays and one weekend) which was taken from patients. The records were analyzed by Nutritionist IV for windows soft ware. Information regarding dietary intake of calories, total carbohydrate, total protein, total fat, fiber and some micronutrients was obtained from the analysis. There were no educational schedules provided for reducing body weight during the course of study.

Biochemical Analyses

Before and after intervention, blood samples were collected after an overnight fasting of 12 hours. The serum of patients were kept at -80°C until biochemical determinations. Serum leptin and ghrelin were measured by enzyme linked immunosorbent assay (ELISA) kits (Boster biological; Ek0437, Phonenix Pharmaceuticals; CEK-031-30 respectively). The immunoplate in these kits are pre-coated with secondary antibody and the nonspecific binding sites are blocked. The secondary antibody can bind to the Fc fragment of the primary antibody (peptide antibody) whose Fab fragment will be competitively bound by both biotinylated peptide and peptide standard or targeted peptide in samples. The biotinylated peptide interacts with streptavidin- horseradish peroxidase (SA-HRP) which catalyzes the substrate solution. The luminescence intensity is directly proportional to the amount of biotinylated peptide-SA-HRP complex but inversely proportional to the amount of the peptide in standard solutions or samples. This is due to the competitive binding of the biotinylated peptide with the standard peptide or samples to the peptide antibody (primary antibody). A standard curve of known concentration can be established accordingly. The unknown concentration in samples can be determined by extrapolation to this standard curve.

Statistical analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS, version 11.5, IL, USA). Because all quantitative parameters had normal distributions according to Kolmogorov-Smirnov Test, data were presented as mean±SD. The paired-t-test was used to compare quantitative parameters at the end of study with baseline. A p-value of < 0.05 was considered statistically significant.

Results

At the end of the 3 months treatment, 80 of the 100 patients (80%) showed a successful eradication of H pylori. Mean body weight, body mass index (BMI), body composition, serum leptin and ghrelin levels of patients at the onset and the end of the study were presented in Table1. The mean body weight (kg), fat mass (kg) and body cell mass (kg) of patients increased after eradication but only the changes of body weight (68.07±1.8 vs 69.38 ±1.9 kg) was statistically significant (P=0.01). As shown in the Table 1, the mean free fat mass (kg) and percentage of free fat mass decreased significantly at the end of study. Although after eradication, the mean resting metabolic rate (kcal/d) and total body water (liter) of subjects reduced but the differences was not statistically significant. *H. pylori* eradication has no significant effect on serum leptin and ghrelin levels (Table 1).

Energy, macro and micro nutrients, and fiber intakes of patients before and after the eradication were shown in Table 2. No significant dietary changes after successful *H. pylori* eradication treatment were found. The only exception was a remarkable enhancement in vitamin C intake (74.8±15.6 vs 90.25±16.3 mg/d) after eradication.

Table 1. The mean body weight, body composition measurements, serum leptin and ghrelin levels of patients before and after eradication (n=80)

	Before eradication	After eradication	*P**
Body weight (kg)	68.07 ± 1.80	69.38 ± 1.90	0.01
Body mass index (kg/m^2)	25.43 ± 0.60	25.93 ± 0.65	0.05
Free fat mass (kg)	46.86 ± 2.90	42.50 ± 3.50	0.04
Free fat mass (%)	77.07 ± 3.40	69.9 ± 3.6	0.03
Fat mass (kg)	15.82 ± 2.40	17.71 ± 2.50	0.18
Fat mass (%)	23.10 ± 3.50	26.77 ± 6.50	0.39
Resting metabolic rate (kcal/d)	1540.41 ± 84.26	1416.20 ± 58.40	0.26
Total body water (liter)	41.2 ± 4.4	37.60 ± 3.39	0.36
Total body water (%)	65.47 ± 5.20	51.00 ± 4.00	0.01
Body cell mass (kg)	22.50 ± 1.20	23.14 ± 1.20	0.19
Leptin (ng/mL)	13.66 ± 5.65	13.22 ± 5.33	0.35
Ghrelin (ng/mL)	20.76 ± 5.00	33.79 ± 12.10	0.46

**P* in comparison with before eradication, by paired t-test.

Table 2: The mean energy, macro and micro nutrients, and fiber intakes of patients before and after eradication (n=80)

	Before eradication	After eradication	*P**
Energy (kcal/d)	1991.52 ± 101.80	1943.55 ± 125.60	0.70
Carbohydrate (gr/d)	305.74 ± 19.20	292.97 ± 28.80	0.44
Carbohydrate % of energy	61.50 ± 1.03	60.37 ± 3.04	0.34
Protein (gr/d)	74.97 ± 3.80	70.58 ± 1.50	0.44
Protein % of energy	15.59 ± 0.54	15.29 ± 0.86	0.87
Fat (gr/d)	50.86 ± 2.20	52.53 ± 5.45	0.66
Fat % of energy	22.90 ± 0.77	24.30 ± 0.85	0.24
Fiber (gr/d)	2.22 ± 0.42	2.62 ± 0.23	0.80
Thiamin (mg/d)	1.60 ± 1.01	1.50 ± 0.60	0.55
Riboflavin (mg/d)	1.13 ± 0.66	1.00 ± 0.10	0.76
Pyridoxin (mg/d)	0.88 ± 0.30	0.55 ± 0.08	0.36
Vitamin C (mg/d)	74.80 ± 7.79	90.25 ± 8.50	0.03

**P* in comparison with before eradication, by paired t-test.

Discussion

Eradication of *H. pylori* improves gastritis [23,24] and decreases the recurrence rate of peptic ulcer disease [3,25]. Most research is recently focused on the relationship between obesity and *H. pylori* infection. The results of our study showed that body weight and BMI of patients increased after eradication. The findings of present study are consistent with the report of previous studies that *H. pylori* infection is inversely related to obesity. For example, Wu et al. [26] reported that the seropositivity of *H. pylori* infection was significantly lower in morbidly obese patients [25]. Furuta et al. [9] have shown the body weight gain after *H. pylori* cure.

At the end of study,the mean free fat mass (kg) and percentage of free fat mass decreased significantly while the mean fat mass and percentage of fat mass increased insignificantly. After eradication, the mean resting metabolic rate (kcal/d) and total body water (liter) of subjects reduced but the differences was not statistically significant.

As ghrelin and leptin are mainly synthesized and secreted by gastric mucosa, it has been postulated that the converse effect of H pylori infection on body weight may ascribe to the difference of plasma ghrelin and leptin levels in patients with or without H pylori infection [24]. On the basis of this hypothesis, the elevated of gastric ghrelin production after *H. pylori* cure may increase serum ghrelin concentration which may stimulate food intake, decreases energy expenditure, and promotes weight gain [27] .On the other hand, successful *H. pylori* eradication can reduce secretion of leptin hormones from stomach. There is evidence that ghrelin and leptin exert opposite actions in nutrient intake and metabolic balance [27,28]. Therefore, it seems that *H. pylori* can play a role in the regulation of leptin and ghrelin expression [15, 21].

The results of present study showed that serum leptin decreased and ghrelin increased insignificantly after eradication. The results of previous studies indicated that serum leptin and ghrelin increased significantly following *H. pylori* eradication [21,29]. In contrast to our results, Gokcel et al. noted that *H. pylori* infection has no significant on plasma ghrelin concentration [20]. We thought that an inconsistency in published results about the effect of *H. pylori* on serum leptin and ghrelin may be due to subject's age, medications, and extent of gastric inflammation.

There are few publications on dietary intake changes after *H. pylori* eradication. In the present study no significant dietary changes after successful *H. pylori* eradication treatment were found. These results are in agreement with data published by Sobczak et al. who noted that dietary intake of patients did not change after *H. pylori* eradication [30]. There was a remarkable enhancement in vitamin C intake (74.8±7.9 vs 90.25±8.3 mg/d) after eradication. Possible explanation to our findings might be that before eradication most of the patient complained of some gastrointestinal symptoms with consumption of fruits and vegetables, whereas enhancement in vitamin C intake at the end of study which may be due to positive effect of successful eradication on improvement of these symptoms.

In this study, we did not compare the biochemical and anthropometric data of *H. pylori* positive patients with *H. pylori* negative subjects this could be the weakness of the study.

In conclusion, the results of this study showed that eradication of *H. pylori* lead to a significant net increase of body weight and decrease free fat mass in patients while dietary intake, serum ghrelin and leptin levels did not change after treatment. It seems that enhanced incidence of gastro-esophageal reflux disease after eradication may be due to increased body weight of these patients. Therefore dietary consulting before and after eradication can be helpful in *H. pylori* infected patients for preventing of weight gain after successful *H. pylori* eradication. However, it would be worthwhile determining the effect of *H. pylori* eradication on other hormones that effect appetite such as insulin or GIP.

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