ARTÍCULO ORIGINAL

Effect of *Helicobacter pylori* infection on gastric ghrelin expression and body weight

Nahum Méndez-Sánchez,* Raul Pichardo-Bahena,* Francisco Vásquez-Fernández, * Jezer I. Lezama-Mora,* Ana L. León-Canales,* Blanca Barredo-Prieto,* Daniela González-Avila,* Guadalupe Ponciano-Rodríguez,** Misael Uribe*

** Departments of Biomedical Research, Gastroenterology & Liver Unit, Medica Sur Clinic & Foundation, Mexico City, Mexico. ** Faculty of Medicine, National Autonomous University of Mexico (UNAM). Circuito Interior s/n, B building, first floor, Ciudad Universitaria, 04510, Mexico City, Mexico*

Correspondence: Nahum Méndez-Sánchez, M.D., Ph.D. Departments of Biomedical Research. Gastroenterology & Liver Unit, Medica Sur Clinic and Foundation. Puente de Piedra 150, Col. Toriello Guerra, Mexico City, Mexico. Phone: (+525) 606-6222, ext 4215 Fax (+525) 666-4031 and 606-1651; e-mail: nmendez@medicasur.org.mx

Disclosure of financial support: Supported by Medica Sur Clinic and Foundation.

Received for publication: January 17th, 2008.
Accepted for publicacion: March 13th, 2008.

SUMMARY Background & Aim: *Ghrelin is a peptide mainly produced by gastric tissue playing an important role in energy homeostasis. It has been suggested that inflammatory and atrophic events induced by* Helicobacter pylori (H. pylori) *infection in gastric mucosa compromise the survival of the ghrelin-producing cells. The aim of this study was to investigate the effect of* H. pylori *infection on gastric ghrelin expression and body weight.* ***Methods:*** *Consecutive patients referred for upper endoscopy were invited to participate. Patients with* H. pylori *infection (determined by histology) were defined as cases and patients without infection as controls. The density of colonization was classified in mild, moderate, or severe infection. Body mass index (BMI) was calculated. Ghrelin-immunoreactive cells were quantified in gastric biopsies by immunohistochemical staining.* ***Results:*** *We studied 189 cases (92 males, 97 females) and 94 controls (55 males, 39 females). Cases were older (48.16 ± 16.44* vs. *42.88 ± 17.04 years,* p *< 0.05) and exhibited a lower percentage of ghrelin-immunoreactive cells (2.13%* vs. *10.43%,* p *< 0.05) than controls. The prevalence of obesity was significantly lower than normal-weight among all cases, independently of the severity of infection (mild infection, 17.6%* vs. *47.3%,* p *= 0.001; moderate-severe infection, 10.4%* vs. *50%,* p *= 0.001). Univariate analysis showed a non-significant trend suggesting a protective effect of* H. pylori *against obesity. Nevertheless, BMI did not differ significantly between cases and controls.* ***Conclusion:*** *Chronic* H. pylori *infection contributes to a lower percentage of gastric ghrelin-immunoreactive cells but has no effect on the body weight of infected patients.*

RESUMEN Introducción y Objetivo: *la ghrelina es un péptido producido principalmente por el tejido gástrico, el cual participa de forma importante en la homeostasis energética. Se ha sugerido que la infección por* Helicobacter pylori (H. pylori) *induce eventos inflamatorios y atróficos en la mucosa gástrica, comprometiendo la supervivencia de las células productoras de ghrelina. El objetivo de este estudio fue investigar los efectos de la infección por* H. pylori *en la expresión gástrica de ghrelina y el peso corporal.* ***Métodos:*** *se invitaron a participar a todos aquellos pacientes referidos para la realización de panedoscopia. Los casos fueron definidos como aquellos pacientes con infección por* H. pylori *(determinada por histología) y aquéllos sin infección fueron definidos como controles. La densidad de la colonización por* H. pylori *fue clasificada en leve, moderada y severa. El Índice de Masa Corporal fue calculado. El número de células inmunorreactivas a ghrelina fue cuantificado en las biopsias gástricas a través de inmunohistoquímica.* ***Resultados:*** *estudiamos 189 casos (92 masculinos, 97 femeninos) y 94 controles (55 masculinos, 39 femeninos). Los casos fueron de mayor edad (48.16 ± 16.44* vs. *42.88 ± 17.04 años,* p *< 0.05) y mostraron un porcentaje menor de células inmunorreactivas a ghrelina (2.13%* vs. *10.43%,* p *< 0.05) que los controles. La prevalencia de obesidad fue significativamente menor que la prevalencia de peso normal en todos los casos, independientemente de la severidad de la infección (infección leve, 17.6%* vs. *47.3%,* p *= 0.001; infección moderada-severa, 10.4%* vs. *50%,* p *= 0.001). El análisis univariado mostró una tendencia no significativa que sugiere un efecto protector del* H. pylori *contra la obesidad. Sin embargo, el IMC no fue diferente entre casos y controles.* ***Conclusiones:*** *la infección crónica*

por H. pylori *contribuyó al menor porcentaje de células inmunorreactivas a ghrelina en tejido gástrico, pero esto no tuvo efectos en el peso corporal de los pacientes infectados.*

Key words: *Obesity,* Helicobacter pylori, *Ghrelin, gastritis, overweight.*

Palabras clave: *Obesidad,* Helicobacter pylori, *ghrelina, gastritis, sobrepeso.*

INTRODUCTION

Obesity is a chronic disease that represents the main risk factor for development of type 2 diabetes mellitus, metabolic syndrome, cardiovascular disease, and other diseases.[1] Its prevalence is increasing throughout the world. In Mexico, data from National Health and Nutrition Survey 2006 shows a prevalence over 30% for obesity and rising to 70% if we combined the prevalence of overweight and obesity.[2]

Body weight is regulated by central mechanisms that controls caloric intake and energy expenditure through neuroendocrine hormones and peripheral markers, which circulate in proportion of body fat content.[3] Obese patients display an imbalance in energy homeostasis. Some of the most important peripheral markers are leptin, insulin, and ghrelin.[4] Ghrelin is a peptide recently discovered by Kojima *et al.*, structurally related to motilin.[5-7] This peptide is primarily produced by X/A-like neuroendocrine cells, which represents approximately 20% of cells in the oxyntic glands. Thus, the stomach is the main source of circulating ghrelin.[8,9] In addition, ghrelin also participates in gastric motility and regulates its own secretion in gastric tissue.[9] Ghrelin regulates satiety by activating hypothalamic neurons that express the agouti-related peptide and neuropeptide Y. Exogenous ghrelin administration to healthy volunteers enhances appetite and increases food intake.[10]

Helicobacter pylori (H. pylori) is one of the most prevalent human infections representing the major cause of chronic gastritis.[11] Its prevalence in developed countries is rare in children, and less than 50% of the population is infected at age 60.[12] In contrast, in developing countries more than 20% of children younger than 5 years are infected, rising to 80% at age 25.[13-15] In Mexico, nearly 60% of the population is infected. Several risk factors for *H. pylori* infection have been identified, and these increase the risk of infection mainly in childhood. These factors include age, low socioeconomic status, crowding, and a low level of education.[16,17] It has been suggested that *H. pylori* infection induces inflammatory and atrophic events in the gastric mucosa. Isomoto *et al*.[18] have reported that ghrelin biosynthesis is affected by *H. pylori* infection, trough compromise functionality and survival of the X/A-like neuroendocrine cells in the oxyntic glands. The aim of our study was to investigate the effect of gastric *H. pylori* infection on ghrelin-expressing cells and body weight.

METHODS

Population and sample

Sample population included consecutive patients who underwent upper gastrointestinal endoscopy for dyspeptic symptoms between January 2005 and July 2006; both men and women between 18 to 60 years were invited to participate.

This prospective study was approved by the Human Subjects Committee of the Medica Sur Clinic and Foundation in accordance with the 1983 Declaration of Helsinki, and written informed consent was obtained from all participants before entry into the study. Exclusion criteria were pregnancy, endocrine disease, previous gastrointestinal surgery, use of medication against *H. pylori* infection during the previous six months, alcohol abuse, or recent treatment with nonsteroidal anti-inflammatory agents.

Physical examination

Body weight was measured to the nearest 0.10 kg. Subjects wore light clothing and no shoes. Height was measured to the closest 0.5 cm. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. BMI was analyzed as a continuous variable by assigning participants as normal weight (BMI < 25 kg/m^2), overweight (BMI 25-29.9 kg/m^2), or obese (BMI ≥ 30

kg/m^2). Other variables included in the study were age and sex.

Histology

Two biopsies were taken during the upper endoscopy from the gastric antrum 2 cm proximal to the pylorus, and two biopsies were taken from the middle portion of the corpus along the greater curvature. Samples were fixed in 10% formalin and embedded in paraffin for histopathological and immunohistochemical assessment.

For histopathological analysis, biopsies were stained with hematoxylin & eosin, and Warthin-Starry. The degree of gastritis activity was determined according to Houston's classification.[11] *H. pylori* chronic infection was determined by histology. Patients with chronic gastritis and *H. pylori* infection were defined as cases and patients with chronic gastritis without the presence *H. pylori* infection were defined as controls. The density of *H. pylori* colonization was determined semiquantitatively as follows: mild infection, small number of *H. pylori* distributed focally; moderate infection, large number of *H. pylori* distributed focally or small number distributed diffusely; severe infection, large number of *H. pylori* distributed diffusely, according to criterion previously reported.[19] Histological evaluations were made by a single expert pathologist without knowledge of the clinical symptoms or endoscope results.

Immunohistochemical analysis

To investigate the effects of chronic *H. pylori* infection on gastric ghrelin-expressing cells, we quantified the percentage of ghrelin-immunoreactive cells in gastric biopsies from cases –selected according to the density of *H. pylori* colonization– and controls, and comparisons were made. Immunohistochemical staining was performed with polyclonal anti-human ghrelin as the primary antibody to quantify the presence of ghrelin in gastric tissue. Immunoreactivity was defined as a brown-ochre stain. The samples were photographed under a light microscope, and the percentage of positive pixels for each field was evaluated using a computerized image analysis program (ImageProPlus Software, V. 4.5.1.22; Media Cybernetics, Silver Spring, MS) as described in the user's manual.

Statistical analysis

Data are expressed as mean ± SD. Variables were compared between cases and controls using the Mann–Whitney U test. The prevalence of overweight and obesity was compared between cases and controls, and also between cases according to the density of *H. pylori* colonization. Correlations were evaluated by linear regression and by calculating the correlation coefficient (r). Odds ratios (ORs) were derived for the exponential of *r* and 95% confidence intervals (CIs) were calculated. A *p* value < 0.05 was considered statistically significant. All statistical analyses were conducted using the statistics program, SPSS/PC v 12.0 (Chicago, IL, USA).

RESULTS

We studied 189 cases (48.7% male, 51.3% female) and 94 controls (58.5% male, 41.5% female). According to the density of *H. pylori* colonization, cases were classi-

TABLE 1
CLINICAL AND ANTHROPOMETRIC DATA OF THE SAMPLE POPULATION

Variable	Cases (*n* = 189)	Controls (*n* = 94)	p
Age (years)	48.16 ± 16.44	42.88 ± 17.04	0.01
Sex			
Men, *n* (%)	92 (48.7)	55 (58.5)	NS
Women, *n* (%)	97 (51.3)	39 (41.5)	NS
Weight (kg)	70.47 ± 69.16	69.16 ± 13.85	NS
Height (m)	1.66 ± 0.09	1.65 ± 0.09	NS
BMI (kg/m^2)	24.93 ± 4.45	24.93 ± 4.56	NS
BMI > 25, *n* (%)	98 (51.9)	43 (45.7)	NS
BMI > 30, *n* (%)	29 (15.3)	16 (17.0)	NS

Abbreviations: BMI, body mass index; NS, not significant.

TABLE 2
DISTRIBUTIONS OF OVERWEIGHT AND OBESITY ACCORDING TO THE DENSITY OF *H. PYLORI* COLONIZATION

H. pylori	*n*	BMI < 25 *n* (%)	BMI 25–29.9 *n* (%)	BMI ≥ 30 *n* (%)	*p*
No infection	94	51 (54.3)	27 (28.7)	16 (17.0)	0.001
Infection					
Mild	131	62 (47.3)	46 (35.1)	23 (17.6)	0.001
Moderate-Severe	58	29 (50)	23 (39.6)	6 (10.4)	0.001

Abbreviation: BMI, body mass index

fied as follows: 131 mild, 44 moderate and 14 with severe infection.

The clinical and anthropometric characteristics of the sample population are shown in *table 1*. Cases were older (48.16 ± 16.44 years *vs.* 42.88 ± 17.04 years, $p < 0.05$) than controls. No differences were observed on gender distribution, body weight or BMI.

Table 2 shows the distribution of obesity, overweight and normal-weight in the cases according to the density of *H. pylori* colonization. All groups shown a lower prevalence of obesity than normal-weight (mild infection, 17.6% *vs.* 47.3%, $p = 0.001$; moderate-severe infection, 10.4% *vs.* 50%, $p = 0.001$). Univariate analysis showed a non-significant trend suggesting a protective effect of *H. pylori* against obesity (Table 3).

Immunohistochemical analysis

The percentage of ghrelin-immunoreactive cells was evaluated in gastric biopsies from 13 patients, 9 cases (two with mild infection, one with moderate infection, and six with severe infection) and 4 controls. Histological analysis determined that none of the gastric biopsies from control group had intestinal metaplasia or atrophy, with mild infiltration of chronic inflammatory cells in all biopsies. In the other hand, cases with mild to moderate *H. pylori* infection also exhibited moderate atrophy with moderate infiltration of chronic inflammatory cells in one patient, and mild to moderate chronic inflammation in two samples. Finally, cases with severe *H. pylori* infection exhibited mild to moderate atrophy in 3 cases, with moderate to marked infiltration of chronic inflammatory cells in the six patients.

Morphometric analysis revealed that cases exhibited lower percentage of ghrelin-immunoreactive cells (2.13% *vs.* 10.43%, $p < 0.05$) than controls. The percentage of ghrelin-immunoreactive cells was negatively correlated with the severity of *H. pylori* infection *(Figure 1)*. We also observed that controls were older than cases with severe infection (59.38 ± 19.63 *vs.* 53.80 ± 19.63 years; $p < 0.05$) but had similar BMI (24 ± 5.23 kg/m^2 *vs.* 23 ± 3.21 kg/m^2).

TABLE 3
TREND OF *HP* INFECTION TO PROTECT AGAINST OBESITY IN UNIVARIATE ANALYSIS.

H. pylori	OR 95%CI
Presence of infection	0.88 (0.45–1.72)
Mild	1.04 (0.51–2.09)
Moderate	0.62 (0.21–1.83)
Severe	0.37 (0.46–3.07)
Moderate–severe	0.56 (0.21–1.53)

Abbreviations: OR, odds ratio; CI, confidence interval. The 95% CIs were not significant.

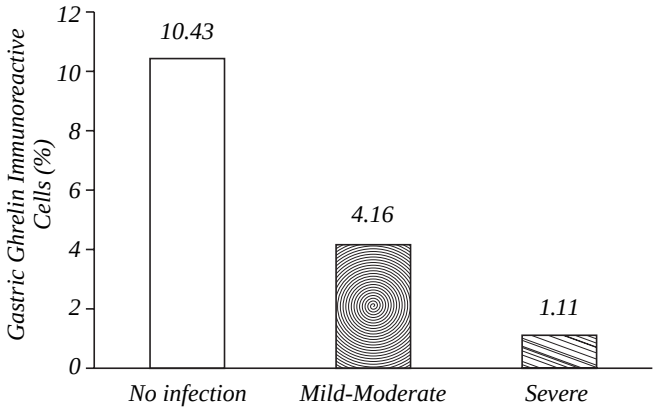

Figure 1. *Negative correlation between the percentage of ghrelin-immunoreactive cells and the* Hp *infection status (no, mild-moderate and severe infection).*

DISCUSSION

Ghrelin is a recently described peptide, highly expressed in gastric tissue.[5,8,9] Its role in energy homeostasis has been demonstrated trough regulation satiety by activation of central mechanism.[20] There is strong evidence suggesting that increments in ghrelin serum concentration enhance appetite, food intake, and weight gain.[10,21,22] However, ghrelin serum concentration is lower in obese patients than in non-obese individuals.[23] It is also observed in patients with chronic *H. pylori* infection.[18] Interestingly, obesity and *H. pylori* chronic infection are two highly prevalent diseases among Mexican population (34% and 60%, respectively); thus, a possible link between both diseases was investigated. We found no relationship between obesity and *H. pylori* chronic infection. A significant low prevalence of obesity was observed initially among cases, according to severity of infection, suggesting a protective role of *H. pylori* infection against obesity. However, the same pattern of distribution was observed in the control group.

An interesting finding of our study was the negative relationship between the density of *H. pylori* colonization and ghrelin-immunoreactive cells. Others reports have found a relationship between the *H. pylori* infection and gastric ghrelin dynamics.[18,25-28] However, the effect of increased density of *H. pylori* on ghrelin expression has not been studied before. This association is supported by previous reports. Grgov *et al.* have reported that a greater density of *H. pylori* colonization along with a tendency toward higher gastritis activity scores in the gastric antrum and gastric corpus.[27] In addition, Isomoto *et al.* found a significant relationship between the progression of histological severity of glandular atrophy and chronic inflammation in the gastric mucosa with lower gastric ghrelin mRNA expression, density of ghrelin-immunoreactive cells, and ghrelin serum concentrations.[24] Liew *et al.* found that the density of ghrelin-positive cells decreased in obese patients with the progression of histological severity of chronic inflammation, neutrophil activity, and glandular atrophy in the corpus.[28] These data suggest that the lower number of ghrelin-expressing cells observed in our study might reflect increased inflammatory and atrophic events induced by the high density of *H. pylori* colonization of the gastric mucosa. This may also explain the negative correlation in our population between the severity of *H. pylori* colonization and percentage of ghrelin-immunoreactive cells.

Finally, our results established no protective role of *H. pylori* chronic infection against obesity. This is an important finding because some authors have suggested that high plasma ghrelin concentration in *H. pylori*-negative patients contributes to increased obesity in developed countries, where the prevalence of chronic *H. pylori* infection is low; in other words, one can infer a possible protective effect of *H. pylori* infection against obesity. Epidemiological data from Mexican population do not support this statement. Despite the recent increase in the prevalence of obesity, the prevalence of chronic *H. pylori* infection has not decreased. Data from the National Health Survey 2000 showed a prevalence of 24.4% of obesity in the Mexican population,[29] rising to 34% in the last National Health and Nutrition Survey 2006.[2] These data confirm that chronic *H. pylori* infection does not provides protection against developing obesity, at least in the Mexican population.

Our study does not clarify the effects of early stages of *H. pylori* infection on gastric ghrelin expression and body weight. Suzuki *et al.*[30] performed an interesting study in Mongolian gerbils inoculated with *H. pylori*., where serum ghrelin concentration, gastric ghrelin expression, and body weight were measured in inoculated and control animals at weeks 4, 17, and 23 after inoculation.[27] Preproghrelin mRNA expression and the number of ghrelin-immunoreactive cells were significantly lower at 17 and 23 weeks in gerbils infected with *H. pylori* than in controls. However, fasting plasma total ghrelin concentration was significantly higher at 17 weeks, and fasting plasma active ghrelin concentration was higher at 17 and 23 weeks in the inoculated gerbils. Interestingly, body weight was greater in the infected than in the control gerbils at 23 weeks (84.1 g *vs.* 79 g). The authors suggested that the higher serum ghrelin concentration was caused by enhanced degranulation of the ghrelin-producing A-like cells induced by inflammatory stimuli, probably through an increase in *H. pylori*-associated tumor necrosis factor-α. Thus, an effect of current *H. pylori* infection on gastric ghrelin expression and body weight cannot be ruled out, and further investigation should clarify these relationships.

In conclusion, our study found that chronic *H. pylori* infection contributes to a lower percentage of gastric ghrelin-immunoreactive cells but has no effect on the body weight of infected patients.

REFERENCES

1. Villa AR, Escobedo MH, Méndez-Sánchez N. Estimates and trends of obesity prevalence through mortality rates associated of chronic diseases in Mexico. *Gac Med Mex* 2004; 140(Suppl. 2): S21-S5.

2. Instituto Nacional de Salud Pública. Encuesta Nacional de Salud y Nutrición 2006. Cuernavaca, Morelos, Mexico.
3. Cummings DE, Schwartz MW. Genetic and pathophysiology of human obesity. *Annu Rev Med* 2003; 54: 453-71.
4. St-Pierre DH, Wang L, Tache Y. Ghrelin: a novel player in the gut-brain regulation of growth hormone and energy imbalance. *News Physiol Sci* 2003; 18: 242-6.
5. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 1999; 402: 656-60.
6. Tomasetto C, Karam SM, Ribieras S, Masson R, Lefebvre O, Staub A, et al. Identification and characterization of a novel gastric peptide hormone: the motilin-related peptide. *Gastroenterology* 2000; 119: 295-405.
7. Méndez-Sánchez N, Chávez-Tapia NC, Uribe M. Ghrelin and the gastrohypothalamic axis. *Gac Med Mex* 2006; 142: 49-58.
8. Date Y, Kojima M, Hosoda H, Sawaguchi A, Mondal MS, Suganuma T, et al. Ghrelin, a novel growth hormone – releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. *Endocrinology* 2000; 141: 4255-61.
9. Ariyasu H, Takaya K, Tagami T, Ogawa Y, Hosada K, Akamizu T, et al. Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. *J Clin Endocrinol Metab* 2001; 86: 4753-8.
10. Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, et al. Ghrelin enhances appetite and increases food intake in humans. *J Clin Endocrinol Metab* 2001; 86: 5992-5.
11. Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. *Am J Surg Pathol* 1996; 20: 1161-81.
12. O'Connor H, Sebastian S. The burden of helicobacter pylori infection in Europe. *Aliment Pharmacol Ther* 2003; 18(Suppl. 3): 38-44.
13. Graham DY, Adam E, Reddy GT, Agarwal JP, Agarwal R, Evans DJ, et al. Seroepidemiology of Helicobacter pylori infection in India. *Dig Dis Sci* 1991; 36: 1084-8.
14. Holcombe C, Omotara BA, Eldrige J, Jones DM. H. pylori, the most common bacterial infection in Africa: a random serological study. *Am J Gastroenterol* 1992; 87: 28-30.
15. Mitchell HM. The epidemiology of Helicobacter pylori. *Curr Top Microbiol Immunol* 1999; 241: 11-30.
16. Graham DY, Malaty HM, Evans DG, Evans DJ, Klein PD, Adam E. Epidemiology of Helicobacter pylori in an asymptomatic population in the United States. Effect of age, race, and socioeconomic status. *Gastroenterology* 1991; 100: 1495-501.
17. Brown LM. Helicobacter pylori: Epidemiology and routes of transmission. *Epidemiol Rev* 2000; 22: 283-97.
18. Isomoto H, Nakazato M, Ueno H, Date Y, Nishi Y, Mukae H, et al. Low Plasma Ghrelin Levels in Patients with Helicobacter pylori – Associated Gastritis. *Am J Med* 2004; 117: 429-32.
19. Talamini G, Zamboni G, Cavallini G. Antral mucosal Helicobacter pylori infection density as a risk factor of duodenal ulcer. *Digestion* 1997; 58: 211-7.
20. Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, et al. A role for ghrelin in the central regulation of feeding. *Nature* 2001; 409: 194-8.
21. Nwokolo CU, Freshwater DA, O'Hare P, Randeva HS. Plasma ghrelin following cure of Helicobacter pylori. *Gut* 2003; 52: 637-40.
22. Wren AM, Small CJ, Ward HL, Murphy KG, Dakin CL, Taheri S, et al. The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. *Endocrinology* 2000; 141: 4325-8.
23. Tschöp M, Weyer C, Tataranni PA, Devanarayan E, Ravussin E, Heiman ML. Circulating ghrelin levels are decreased in human obesity. *Diabetes* 2001; 50: 707-9.
24. Isomoto H, Ueno H, Saenko VA, Mondal MS, Nishi Y, Kawano N, et al. Impact of Helicobacter pylori infection on gastric and plasma ghrelin dynamics in humans. *Am J Gastroenterol* 2005; 100: 1711-20.
25. Tatsuguchi A, Mikaye K, Gudis K, Futagami S, Tsukui T, Wada K, et al. Effect of Helicobacter pylori infection on ghrelin expression in human gastric mucosa. *Am J Gastroenterol* 2004; 99: 2121-7.
26. Osawa H, Kita H, Ohnishi H, Nakazato M, Date Y, Bowlus CL, et al. Changes in plasma ghrelin levels, gastric ghrelin production, and body weight after Helicobacter pylori cure. *J Gastroenterol* 2006; 41: 954-61.
27. Grgov S, Stefanovie M, Katie V. The relationship between the density of Helicobacter pylori colonization and the degree of gastritis severity. *Arch Gastroenterolhepatol* 2002; 21: 3-4.
28. Liew PL, Lee WJ, Lee YC, Chen WY. Gastric ghrelin expression associated with Helicobacter pylori infection and chronic gastritis in obese patients. *Obese Surg* 2006; 16: 612-9.
29. Olaiz-Fernández G, Rojas R, Barquera S. Encuesta Nacional de Salud 2000. Cuernavaca, Morelos, México
30. Suzuki H, Masaoka T, Hosada H, Ota T, Minegishi Y, Nomura S, et al. Helicobacter pylori infection modifies gastric and plasma ghrelin dynamics in Mongolian gerbils. *Gut* 2004; 53: 187-94.