Trends in Medical Research

ISSN 1819-3587

Academic Journals Inc.

www.academicjournals.com

Trends in Medical Research 8 (2): 63-85, 2013
ISSN 1819-3587 / DOI: 10.3923/tmr.2013.63.85

Effect of *H. pylori* and its Eradication on Gastric Ghrelin Secretion

[1]Mohammed Amin Mohammed and [2]Nesreen Moustafa Omar

[1]Department of Internal Medicine Gastroenterology and Hepatology Unit, Faculty of Medicine, Mansoura University, Egypt

[2]Department of Histology and Cell Biology, Faculty of Medicine, Mansoura University, Egypt

Corresponding Author: Mohammed Amin Mohammed, Department of Internal Medicine Gastroenterology and Hepatology Unit, Faculty of Medicine, Mansoura University, Egypt

ABSTRACT

Helicobacter pylori infection and its eradication may influence plasma levels and gastric production of some peptides, which can affect appetite. Ghrelin, an orexigenic hormone, is primarily produced in and secreted from oxyntic mucosa of the stomach. Ghrelin has been demonstrated to play a central role in appetite, food intake and energy homeostasis. As ghrelin production in human is exclusively gastric origin, it is conceivable and not surprising that any injury to the gastric mucosa will affect plasma ghrelin concentrations. To verify this hypothesis, a total of 135 adult consecutive individuals with normal body mass index including 84 *H. pylori*-infected and 51 *H. pylori*-negative subjects were included in a randomized controlled trial. Gastric ghrelin mRNA expression levels were measured in endoscopic biopsy specimens in both groups before and after *H. pylori* eradication. Also, plasma active n-octanoyl ghrelin and obestatin levels and ghrelin/obestatin ratio were measured in both groups before and after *H. pylori* eradication. The treatment group (44/84) received triple *H. pylori* eradication therapy for 7 days and followed up for 6 months. In contrast to obestatin, plasma and gastric ghrelin mRNA expression levels were significantly lower in *H. pylori*-infected subjects. *H. pylori* eradication significantly reversed these changes. The decrease in plasma ghrelin concentration in *H. pylori*-positive subjects was accompanied by depletion of ghrelin mRNA expression. These findings suggest that *H. pylori* induced chronic gastritis impair gastric ghrelin production and consequently the decrease in plasma ghrelin concentration.

Key words: Ghrelin, obestatin, *Helicobacter pylori*, eradication

INTRODUCTION

Ghrelin is a 28 amino acid peptide hormone discovered by Kojima *et al.* (1999) and produced mainly in the stomach. Human ghrelin producing cells, the P/D_1 enteroendocrine cells of the gastric mucosa (X/A-like cells in rats), are situated mainly in the corpus and fundus of the stomach. Ghrelin is secreted into the extracellular matrix then to the general circulation through capillary vessels in the gastric lamina propria in its active octanoylated form (Date *et al.*, 2000). It has been demonstrated that ghrelin plays central as well as peripheral roles in appetite modulation, food intake, eating behavior, body-weight regulation, energy homeostasis, gastric motility and acid secretion (Masuda *et al.*, 2000; Nakazato *et al.*, 2001).

Ghrelin O-acyltransferase (GOAT) is responsible for octanoylation of ghrelin before its secretion into blood stream which is essential for biological actions of ghrelin. Only the active acylated form of ghrelin can bind to and stimulate the ghrelin receptors which are growth hormone secretagogue receptors (GHS-R) accounting for many of its biological actions such as appetite stimulation and anti-inflammatory actions (Osawa, 2008; Jeffery *et al*., 2011).

Ghrelin, to a lesser extent, was found to be expressed in and secreted from multiple tissues like the gut, kidney, lung, pancreas (A cells) and hypothalamic nucleus (Ariyasu *et al*., 2001). It exerts multiple endocrine, paracrine and autocrine effects. Circulating ghrelin levels have been associated with and responsible for multiple biological effects including appetite and body weight control. Ghrelin stimulates GHS-R and via a GHRH-dependent mechanism will stimulate growth hormone and insulin-like growth factor 1 (ILGF-1) release. The released hormones (GH and ILGF-1) affect a range of physiological processes (Nakazato *et al*., 2001).

The normal serum ghrelin level in adults of healthy body mass index (BMI) ranges from 145-200 fmol mL^{-1} with little higher levels in females. Plasma ghrelin secretion is episodic and dimorphic. Ghrelin is considered to be a short term orexigen related to meals as its plasma concentrations rise before and decrease after meals. Collectively, ghrelin regulates somatic and adipose tissue growth thus contributes to long term regulation of body weight. It has been suggested that ghrelin increases acid secretion, stimulates gastric emptying (Cummings *et al*., 2002) and exerts a gastro-protective actions on the gastric mucosa (Asakawa *et al*., 2001).

It is intuitive that chronic gastritis and gastric damage with or without atrophy might affect ghrelin production, activation and secretion because of the gastric location of ghrelin producing cells. This will affect appetite and food intake and in turn change body weight and BMI (Jeffery *et al*., 2011).

Helicobacter pylori is a gram negative curved or S shaped rods and has a worldwide prevalence. More than 50% of the world's population are infected with higher prevalence in developing countries (Brown, 2000). *H. pylori* specifically infects the gastric mucosal cells. The stomach and/or heterotopic gastric mucosa are the natural habitat of this well adapted parasite (Hofman *et al*., 2004).

Several studies have suggested an association between *H. pylori* infection and various gastric and extra-digestive diseases such as immunological, cardiovascular and a variety of other pathologies (Gasbarrini *et al*., 1999, 2004). *H. pylori* infection leads to multiple gastro-doudenal pathologies (Schreiber *et al*., 2004). *H. pylori* infection is involved in the pathogenesis of gastritis, gastric and doudenal erosions and/or ulcers; mucosa associated lymphoid tissue lymphoma (MALT-oma), intestinal metaplasia with or without dysplasia or gastric adenocarcinoma (Uemura *et al*., 2001; Goodwin *et al*., 1997).

Several sero-epidemiological studies have suggested an association between ghrelin and *Helicobacter pylori* infection. Many authors reported an increase of plasma ghrelin levels after *H. pylori* eradication (Nweneka and Prentice, 2011; Nwokolo *et al*., 2003). On the other hand, others concluded that *H. pylori* infection and/or its eradication had no effect on ghrelin concentrations (Gokcel *et al*., 2003). So, it is not known whether alteration of plasma ghrelin levels is the result of compromised gastric ghrelin production caused by *H. pylori* infection. Consequently, the relationship between gastric ghrelin and *H. pylori* is still conflicting. The aim of this study was

to investigate and assess the effects of *H. pylori* infection on gastric ghrelin mRNA expression using real-time quantitative RT-PCR in gastric endoscopic biopsies and concomitantly examining plasma ghrelin concentrations.

MATERIALS AND METHODS

Subjects: A total of 161 consecutive adult outpatients, older than 20 years, with functional dyspepsia (classification according to Rome III criteria) (Drossman, 2006) were initially enrolled in this study (from June 2010 through 2011). The study was approved by the Ethical Commission and Institutional Review Board of Mansoura University Hospital in EGYPT. A written informed conscious consent was obtained from all patients before their participation.

Exclusion Criteria included chronic or psychiatric illness, age<20 year; age>70 year; pregnancy; liver disease; renal impairment; cardiopulmonary disease; abnormal macroscopic endoscopic lesions, including gastric ulcers, cancer or duodenal ulcers; previous gastrointestinal surgery; history of *H. pylori* eradication; smoking, drug or alcohol abuse-defined as consumption of more than two alcoholic drinks per day and NSAIDs or PPIs administration during the 2-4 week prior to the study. Patients with histopathological changes higher than those of non-atrophic gastritis (according to Sydney System Scale update (Dixon *et al*., 1996) and with a Body Mass Index (BMI) below 18 and above 29 kg m^{-2} were excluded from the study.

Methods

History and examinations: Initially, all patients completed a detailed questionnaire regarding diet and habits, submitted to thorough history taking and detailed physical examinations performed at fasting in the morning. The selected subjects had their BMI calculated (as body weight in kilograms divided by the square of their height in meters (Weigle *et al*., 2003). The appetites of all subjects were estimated using a Visual Analog Scale (VAS) of appetite (Kim *et al*., 2008).

Endoscopy: All the participants underwent routine investigations and upper gastrointestinal endoscopic evaluation at enrolment (a gastroscopy with biopsy; XQ20 or XQ40, Pentax Fibreoptic, Tokyo) performed after an overnight fast between 08:00 and 10:00 a.m. to avoid the effects of diurnal hormone variation.

During endoscopy, the macroscopic picture of the mucosa was assessed in terms of color, mobility, vascular network. The intensity of various features of gastritis was evaluated in accordance with Sydney visual scale of division criteria, with Houston modification for chronic gastritis (Bartuzi *et al*., 2000).

From each patient, four biopsy specimens were taken from the midportion of the fundus along the greater curvature; four were taken from intact mucosa in the gastric antrum, 2 cm proximal to the pylorus and two from the incisura angularis where maximal degrees of atrophy and intestinal metaplasia are consistently found (Stemmermann, 1994).

One biopsy from each site was used to perform either biopsy urease test (CLO test: Ballard Medical Products, Draper, UT, USA) or culture to detect *H. pylori* infection.

One biopsy sample from each site was fixed in neutral formaldehyde 10% solution, dehydrated in ascending grades of alcohol then embedded in paraffin for histological assessment.

Table 1: Flow chart of the study

	Groups			
	H. pylori+ve (84 (36/48))		*H. pylori*-ve (51(22/29)	
Time interval	Treatment gp (Ia)	Control gp (Ib)	Without gastritis	With gastritis
Baseline (n(♀/♂)	44 (18/26)	40 (18/22)	35 (16/19)	16 (6/10)
After two week	42 (two not eradicated)	39 (one received eradication ttt)	35(16/19)	16(6/10)
After one month	41(15/26) (one received drugs which affect appetite)	37(15/22) (two received eradication ttt)	34(15/19) (one received drugs which affect appetite)	15(6/9) (one received ttt for gastritis)
After 6 months	39 (13/26) (two received drugs which affect appetite)	35(16/19) (two received drugs which affect appetite)	-	-

A total of 161 consecutive adult outpatients with Functional Dyspepsia, Twenty six patients were excluded according to exclusion criteria, The remaining 135 patients were assigned to the following groups

The remaining two specimens, taken from the anterior and posterior wall of the antrum or corpus were transferred to tubes containing TRIzol® (Gibco, Long Island, NY, USA) and stored immediately at -70°C until assayed for gastric ghrelin concentrations.

Groups: A total of 161 consecutive adult outpatients with Functional Dyspepsia (FD) were initially enrolled in this study. From these patients, eleven with severe oesophagitis, nine with peptic ulcer, one with gastric cancer and five with atrophic gastritis were excluded. The remaining 135 patients (Group I:84 *H. pylori* positive and Group II:51 *H. pylori* negative) were assigned to the following groups (Table 1):

Group Ia: treatment group: This group comprised 44 *H. pylori* positive patients (females/males 18/26; median age 44 (29-55 years) with a diagnosis of chronic *H. pylori* gastritis. They were re-evaluated one and six months after appropriate eradication therapy which included; Lansoprazole (30 mg bid), Metronidazole (500 mg bid), Amoxicillin (1000 mg bid) and Clarithromycin (500 mg bid) for seven days. This 7-day regimen of quadruple therapy was proved highly (98%) effective eradication therapy (De Boer *et al*., 1996).

Group Ib: Non-treated control group: This group comprised 40 *H. pylori* positive patients (females/males 18/22; median age 44 (29-63 years) with a diagnosis of chronic *H. pylori* gastritis. This control group did not receive any prescription and was directed not to take any medication.

Group IIa: *H. pylori* negative patients without chronic gastritis: This group comprised 35 patients (females/males 16/19; median age 45 (30-57 years) patients with a diagnosis of *H. pylori* negative and a normal gastric histology.

Group IIb: *H. pylori* negative patients with chronic gastritis: This group comprised 16 patients (females/males 6/10; median age 46 (36-57 years) patients with a diagnosis of *H. pylori* negative but with chronic gastritis.

All groups underwent a repeated endoscopy one month later. The eradicated subjects (39/44) were followed up for further six months. All of the procedures were repeated at the time of repeated endoscopy including the determination of gastric and plasma active ghrelin and obestatin concentrations.

Table 2: Definition and grading guidelines for each of the histological features to be graded according to the Sydney classification Kim and Baek (1999) Mild if gastritis score is ≤10, Moderate if gastritis score is 10-20, Severe if gastritis score is ≥20

Feature	Definition	Grading guidelines
H. pylori (HP)	Density of Helicobacter-like organisms overlying epithelium	None:no curved bacilli
		Mild:organisms covering <1/3 of the surface
		Moderate:intermediate numbers
		Severe:organisms clusters over >2/3 of surface
Activity polymorph	Neutrophils polymorph infiltration of the lamina propria,	None:polymorph difficult to find
-nuclear cells (PMN)	pits or surface epithelium	Mild:<1/3 of pit and surface infiltrated
		Moderate:1/2-2/3 of pit and surface infiltrated
		Severe:>2/3 of pit and surface infiltrated
Chronic inflammation	Increase in lymphocytes and plasma cells in the lamina	None:normal lymphocytes and plasma cells
= mononuclear cells	propria	Mild:mild increase in density
(MNC)		Moderate:moderate increase in density
		Severe:severe increase in density
Atrophy	Loss of specialized glands from either antrum or body	None:absent
		Mild:mild loss
		Moderate:moderate loss
		Severe:severe loss

Histology: The biopsy samples were treated in a standard manner. The sections were stained with haematoxylin and eosin for conventional histopathological examination and preliminary assessment of presence of *H. pylori* using oil immersion lens. *H. pylori* infection was further evaluated with (modified) Giemsa staining and/or Cresyl violet acetate stain (Stevens and Francis, 1996). Two paraffin-embedded tissue blocks (from the antrum and corpus) were used for microscopic section preparation. From each paraffin block, two sections were obtained.

The microscopic gastric mucosa assessment, *H. pylori* density, polymorph-nuclear neutrophil activity (PMN) and chronic inflammation (mononuclear cell; MNC) were scored on a scale of 0 to 3 to each graded variable: 0 = absence, 1 = mild, 2 = moderate and 3 = severe degree (Table 2) based on criteria according to the updated Sydney System Classification. The scores from the body were calculated to give a total "corporal gastritis score" for each patient which ranged from 0-15. Similarly, figures from gastric antrum were calculated to give a total "antral gastritis score" for each patient. If the difference between both scores was more than two points, it was considered either antral-predominant or corpus-predominant gastritis. Atrophy and metaplastic changes in gastric glands were excluded (Dixon *et al*., 1996).

Histological evaluations were performed by a well-trained Histopathologist who was blind to the treatment, endoscopic diagnosis, *H. pylori* status and subject group identity.

Hormonal assays: Venous blood samples were collected after an over-night fast into the Lavender Vacutainer tubes (#VT-6450) which contain EDTA-2Na and can collect up to 7 mL blood/tube. Gently rock the Lavender Vacutainer tubes several times immediately after collection of blood for anti-coagulation. Transfer the blood from the lavender vacutainer tubes to centrifuge tubes containing aprotinin (0.6 TIU mL^{-1} of blood) and gently rock several times to inhibit the activity of proteinases. Centrifuge the blood at 1,600×g for 15 min at 4°C and collect the plasma. Plasma stored at -70°C until assayed. 1 TIU (Trypsin Inhibitor Unit) = 1025~1300 KIU (Kallikrein Inhibitor Unit) (Catalog No.: RK-APRO).

Plasma levels of active octanoylated ghrelin were determined with Human Ghrelin (Active) competitive radioimmunoassay (RIA) kit which measures only acylated ghrelin (Linco/Millipore Research, St. Charles, MO, Bio-manufacturing and Life Science Research, Catalogue Number:

GHRA-88 HK). The sample designated for ghrelin analysis was acidified with 50 μL of 1 NHCl and 10 μL of phenylmethylsulfonyl fluoride (PMSF) were added at 1 mL of plasma. These samples were measured in duplicate. Standard Curve Range: 10-2000 pg mL^{-1}. Intra-assay coefficients of variation (CV) were 7.3 and 7.8% at 78 and 538 pg mL^{-1}, respectively. Inter-assay CV was 9 and 12% at 40 and 356 pg mL^{-1}. Accuracy was 100%. Assay sensitivity is 7.8 pg mL^{-1} when using a 0.1 mL sample size. Assay specificity is as follows: human ghrelin, 100%; ghrelin 1-10, 100%.

The plasma obestatin was measured using a radioimmunoassay (RIA) kit (Phoenix Pharmaceuticals, INC.). Catalog # RK-031-92 Range: 5-640 pg $tube^{-1}$ in 100 μL of Standard solution = 50-6400 pg mL^{-1}. Lowest Detection Limit: 10 pg mL^{-1}. The inter-and intra-assay coefficients of variation were less than 11% and less than 5%, respectively (Patrono and Peskar, 1987).

Gastric mRNA detection

RNA isolation: Total RNA was isolated from the biopsy specimen with ISOGEN (Nippon Gene, Tokyo, Japan) using a standard reported procedure. The isolated RNA used for RT-PCR by using random nanomers and reverse transcriptase according to the manufacturer's protocol (Mackey and Chomczynski, 1996).

RNA isolation protocol: The protocol includes the following steps:

- **Homogenization:** ISOGEN 1 mL (50~100 mg tissue) using a glass-Teflon or Polytron homogenizer
- **Phase separation:** Homogenate+0.2 mL chloroform centrifuge at 12000 g (max.)
- **RNA precipitation:** Aqueous phase+0.5 mL isopropanol. Precipitate RNA from the aqueous phase by mixing with 0.5 mL of isopropanol per 1 mL of ISOGEN. RNA precipitate (often invisible before centrifugation) forms a gel-like pellet or white pellet on the side and bottom of the tube
- **RNA wash:** Ethanol 75% 1mL. Remove supernatant and wash the RNA pellet (by vortexing) with 75% ethanol and subsequent centrifugation at 7500 g (max.) at 4~25 °C for 5 min
- **RNA solubilization:** SDS or water 0.5%. Remove the ethanol and briefly air-dry the RNA pellet for 3~5 min. Dissolve RNA in water or 0.5% SDS by passing the solution several times through a pipette and incubate at 55~60°C for 10~15 min. Water or 0.5% SDS solution used for RNA solubilization were made RNase-free by diethylpyrocarbonate (DEPC) treatment

The final preparation of total RNA was highly pure, free of DNA and proteins and had a 260/280 ratio 1.6-1.9. For optimal spectrophotometric measurements, RNA aliquots were diluted with water or buffer with pH>7.5. The concentration and purity of the RNA were determined using spectrophotometry (Ultrospec® 1100 Pro; Amersham Pharmacia Biotech, Buck, UK) to measure the optical density ratio at 260 and 280 nm. The isolated purified RNA is ready to use in, real-time RT-PCR.

REAL-time RT-PCR of ghrelin mRNA: The expression level of ghrelin mRNA was evaluated by using a real-time quantitative RT-PCR method. One microgram of total RNA was used as a template to generate cDNA by using M-MLV (Moloney murine leukemia virus) reverse transcriptase (Super Bio, Suwon, Korea, Cat. No. 04655885001) with random hexamer priming (Car. No. 11034731001). The resultant cDNA was amplified using an Exicycler 96 Real-time Quantitative Thermal Block (Bioneer, Seoul, Korea) then used as a template for RT-PCR.

Real-time PCR analysis was carried out with SYBR® Premix Ex Taq™ (TaKaRa Bio, Tokyo, Japan, Cat. No. RR041A) and gene specific primers retrieved from Primer Bank.

The sequences of primers were as follows: Ghrelin: 5'-ATG CTC CTG TGG GAC TTG-3'(sense) and 5'-TCT GCT TGA CCA CCT TCT T-3'(antisense; Genebank; Accession number NM 016362; ACC No. NM 016362; product size, 155 bp) The PCR specificity is examined by 3% Ready Agarose Precast Gel (Bio-Rad). Analyze the real-time PCR result with the SDS 7000 software. The gene mRNA levels were corrected using **β**-actin.

Determinations of intragastric pH: At gastroscopy, immediately after passing the endoscope into the stomach, a sterile Teflon catheter was passed through the biopsy channel and starting from the mid-body along the greater curve approximately 5 mL of gastric juice were aspirated avoiding contamination with blood and collected in a sterile tube containing EDTA. Gastric juice pH values were immediately measured with a glass electrode pH meter.

Statistical analysis: Data were analyzed using SPSS software (Version 19.0). Quantitative data were expressed as (Mean±SD) while qualitative data were expressed as number and percentage. Continuous data are expressed as median (range) and were evaluated by appropriate statistical tests; t test (for paired data e.g. to compare ghrelin/obestatin ratio in the plasma and gastric mRNA expression measured before and after treatment). Proportions were compared by means of Fisher's exact test. Correlations were evaluated using the Spearman rank correlation coefficient test. Kruskal-Wallis one way analysis of variance (ANOVA) compares more than two groups.

Multiple regression analysis was used to assess the effects of *H. pylori* eradication on ghrelin production by the gastric mucosa, adjusted by the weight change and change in neutrophil or mononuclear cell infiltration. Subgroups (percentages of patients) were compared by using the McNemar test. A value of $p \leq 0.05$ was considered statistically significant. Sensitivity, specificity and predictive values were calculated to study the overall predictability of other techniques (histopathology, urease test ...) according to the following formulae:

$$\text{Positive (+ve) predictive value} = \frac{\text{N}^0 \text{ of true+ve cases}}{\text{N}^0 \text{ of all+ve cases with screening test}} \times 100$$

$$\text{Negative (-ve) predictive value} = \frac{\text{N}^0 \text{ of true-ve cases}}{\text{N}^0 \text{ of all-ve cases with screening test}} \times 100$$

$$\text{Overall predictability (accuracy)} = \frac{\text{N}^0 \text{ of true+ve and true-ve cases}}{\text{Total N0 of all+ve and all-ve cases}} \times 100$$

$$\text{Sensitivity} = \frac{\text{N}^0 \text{ of true+ve cases}}{\text{N}^0 \text{ of all+ve cases with reference test}} \times 100$$

$$\text{Specificity} = \frac{\text{N}^0 \text{ of true-ve cases}}{\text{N}^0 \text{ of all-ve cases with reference test}} \times 100$$

RESULTS

Table 1 shows the flow chart of the study. Twenty six patients of a total of 161 consecutive adult outpatients with Functional Dyspepsia (FD) were excluded according to exclusion criteria. The remaining 135 patients were assigned to the following groups: group Ia: *H. pylori*+ve (84 (♀/♂:

Table 3: Histological Criteria of Gastritis in *H. pylori*+ve Subjects (Group I) Mild if gastritis score is ≤10; Moderate if gastritis score is 10-20; Severe if gastritis score is ≥20; Predominance: if the differences between antral and corporal gastritis scores is >2

	Chronic superficial non atrophic *H. pylori* induced gastritis							
	H. pylori density and colonization				Severity, extent and topography of gastritis			
	N	Severe 40/84	Moderate 33/84	Mild 11/84	Antral restricted 2/84	Corporal restricted 12/84	Diffuse pan gastritis 70/84	Severity
Treatment Gp at baseline	7	-	3	4	1	2	4	Mild
	31	16	15	-		1	30	Moderate
	6	6	-	-		3	3	Severe
	44	22	18	4	1	6	37	Total
Control Gp at baseline	8	-	1	7	1	1	6	Mild
	26	12	14	-		2	24	Moderate
	6	6	-	-		3	3	Severe
	40	18	15	7	1	6	33	Total

36/48) and group Ib: *H. pylori*-ve (51(♀/♂22/29). Only 39 patients in group Ia (13/26) were followed up to 6 ms (two were not eradicated and three received drugs which affect appetite). Five of forty in the control groups were excluded from follow up; three received eradication therapy for *H. pylori* and two received drugs which affect appetite.

Table 2 Shows the Definition and Grading Guidelines for each of the Histological Features Graded According to the Sydney Classification.

Table 3 shows the Histological Criteria of Gastritis in *H. pylori*+Ve Subjects (the type, severity, extent and topography of gastritis and Density of *H. pylori* colonization). All patients of group I had chronic superficial, non atrophic, *H. pylori*-induced gastritis. The majority of patients had diffuse pan gastritis (70/84; 83%) with corporal predominance while only 2/84 had antral restricted gastritis and the remaining 12/84 (14.3%) had corporal restricted gastritis. Most patients had moderate (33/84; 39%) to severe (40/84; 47.6%) *H. pylori* colonization from which only 12/84 (14.3%) had severe *H. pylori* gastritis.

Table 4 shows some characteristics (Mean±SD) of all patients groups including the age, sex, BMI, VAS, total gastritis scores, *H. pylori* density in the corpus, PMN, MNC.

Table 5 shows some characteristics (Mean±SD) of all patients groups including plasma ghrelin and obestatin levels, ghrelin/obestatin ratio, gastric ghrelin mRNA expression level and intragastric pH.

Comparative analysis of the different characteristics in different groups (p-values and ANOVA are significant values if $p \leq 0.05$) are given in both Table 4 and 5. There were no significant differences between the *H. pylori*+ve and *H. pylori*-ve groups were observed with regard to age, sex, BMI, VAS ($P1 \leq 0.05$).While, there were highly significant differences between the two groups with regard to baseline characteristics including plasma ghrelin and obestatin levels, ghrelin/obestatin ratio, gastric ghrelin mRNA expression levels, neutrophil (PMN), mononuclear cell (MNC) infiltration, HP density, total gastritis score and intragastric pH ($P1 \leq 0.01$). The increase in gastritis scores (total, *H. pylori*, PMN, MNC) was associated with significant decrease in plasma and gastric ghrelin levels and ghrelin/obestatin ratio but was associated with significant increase in plasma obestatin level and intragastric pH.

No initial differences between the treatment (Ia) and control (Ib) groups were observed with regard to all baseline characteristics ($P2 \leq 0.05$).

Table 4: Clinical, demographic data and histological criteria of gastritis in all groups of patients

Groups	Age	Sex	BMI	VAS	Total gastritis scores	*H. pylori* density	PMN	MNC
Gp Ia at baseline								
N	44	44(18/26)	44	44	44	44	44	44
M	42.773	0.5909	22.768	54.227	14.886	2.409	2.409	2.159
SD	7.6092	0.4974	2.7897	6.4695	4.6165	0.6583	0.6583	0.5683
Gp Ib at baseline								
N	40	40(18/22)	40	40	40	40	40	40
M	45.275	0.55	23.425	53.375	14.625	2.275	2.275	2.275
SD	9.982	0.5038	2.6129	7.1672	5.2413	0.7506	0.7506	0.7506
Gp Ia at 4 week								
N	41	41(15/26)	41	41	41	41	41	41
M	42.122	0.6341	22.812	55.171	3.3659	0	1.659	2.049
SD	8.7355	0.4877	2.8606	5.8091	2.3426	0	0.6168	0.669
Gp Ib at 4 week								
N	37	37(18/26)	37	37	37	37	37	37
M	46.162	0.5946	23.351	53	15.784	2.351	2.351	2.351
SD	11.354	0.4977	2.5648	6.7454	6.0879	0.7156	0.7156	0.7156
Gp Ia at 6 month								
N	39	39(13/26)	39	39	39	39	39	39
M	42.385	0.6667	27.09	63.615	0.1795	0	0	0
SD	8.4934	0.4776	1.0485	9.9726	0.3888	0	0	0
HP-ve without gastritis (Gp IIa)								
N	35	35(16/19)	35	35	35	35	35	35
M	44.2	0.5429	22.923	54	0	0	0	0
SD	7.9698	0.5054	2.8277	4.8689	0	0	0	0
HP-ve with gastritis (Gp IIb)								
N	16	16(6/16)	16	16	16	16	16	16
M	46.125	0.625	23.781	51.188	8.1875	0	2	1.188
SD	5.9316	0.5	3.2776	5.0229	2.6887	0	0	0.4031
P1	0.517	0.975	0.824	0.523	0.001	0.001	0.001	0.001
P2	0.198	0.709	0.27	0.568	0.809	0.386	0.386	0.425
P3	0.081	0.724	0.386	0.131	0.001	0.001	0.001	0.057
P4	0.715	0.687	0.943	0.482	0.001	0.001	0.001	0.414
P5	0.716	0.697	0.901	0.814	0.373	0.978	0.65	0.65
ANOVA 1	0.935	0.652	0.001	0.001	0.001	0.001	0.001	0.001
ANOVA 2	0.203	0.293	0.001	0.26	0.001	-	-	0.001

MNC: Mononuclear cells, PMN: Polymorphonuclear cells, VAS: Visual analogue scale, BMI: Body mass index, P1: Compares baseline characteristics of HP+ve and HP-ve groups, P2: Compares baseline characteristics of group Ia (treatment) and group Ib (control), P3: Compares characteristics of group Ia (treatment) and group Ib (control) after one month. P4: Compares characteristics of group Ia (treatment) at baseline and after one month, P5: Compares characteristics of group Ib (control) at baseline and after one month, Anova 1: Compares characteristics of treatment group at baseline, one month and 6 months, Anova 2: Compares baseline characteristics of HP+ve group according to density of *H. pylori*, Significance if $p \leq 0.05$

After one month follow up, there were highly significant differences between the treatment (Ia) and control (Ib) groups with regard to characteristics including plasma ghrelin and obestatin levels, ghrelin/obestatin ratio, gastric ghrelin mRNA expression levels, PMN, HP density, total gastritis score and intragastric pH ($P3 \leq 0.01$). Whereas, no differences between the two groups were observed with regard to age, sex, BMI, VAS and MNC infiltration ($P3 \leq 0.05$).

Table 5: Laboratory parameters in all groups of patients

Group (Gp)	Ghrelin	Obestatin	Ghrelin/obestatin ratio	Gastric ghrelin mRNA	Intragastric pH
Treatment (Gp Ia) baseline					
Mean	268.49	127.159	2.225	67	5.7955
SD	83.215	16.4423	0.3942	21.546	1.2682
Control (Gp Ib) at baseline					
Mean	265.77	126.823	2.313	59.843	6.2105
SD	85.819	17.0265	0.4227	15.368	1.008
Treatment (Gp Ia) at 4 week					
Mean	426.07	119.321	3.593	467.29	3.8439
SD	89.371	15.2845	0.5714	77.075	0.8295
Control (Gp Ib) at 4 week					
Mean	240.09	131.56	2.28	54.297	5.5941
SD	69.914	16.2671	0.3685	19.411	1.5752
Treatment (Gp Ia) at 6 m					
Mean	455.87	118.795	3.724	492.36	3.3385
SD	54.421	15.8582	0.5518	105.56	1.203
HP-ve without gastritis (Gp Iia)					
Mean	520.91	108.629	5.139	522.09	3.7986
SD	89.531	8.5855	0.5878	81.208	0.3482
HP-ve with gastritis (Gp Iia)					
Mean	506.88	112.4	5.094	491	3.9344
SD	61.696	10.2944	0.4106	60.485	0.416
P1	0.001	0.001	0.001	0.001	0.001
P2	0.883	0.927	0.324	0.086	0.103
P3	0.001	0.001	0.001	0.001	0.001
P4	0.001	0.026	0.001	0.001	0.001
P5	0.156	0.217	0.713	0.167	0.11
ANOVA 1	0.001	0.028	0.001	0.001	0.001
ANOVA 2	0.001	0.001	0.001	0.001	0.001

P1: Compares baseline characteristics of HP+ve and HP-ve groups, P2: Compares baseline characteristics of group Ia (treatment) and group Ib (control), P3: Compares characteristics of group Ia (treatment) and group Ib (control) after one month, P4: Compares characteristics of group Ia (treatment) at baseline and after one month, P5: Compares characteristics of group Ib (control) at baseline and after one month, Anova 1: Compares characteristics of treatment group at baseline, one month and 6 months, Anova 2: Compares baseline characteristics of HP+ve group according to density of *H. pylori*, Significance if $p \leq 0.05$

After one month follow up, no statistically significant differences between the subjects of control (Ib) groups were observed with regard to all characteristics ($P5 \leq 0.05$).While, in treatment group, at one month after eradication therapy, gastritis scores significantly improved (total, *H. pylori*, PMN) and consequently plasma and gastric ghrelin levels and ghrelin/obestatin ratio were significantly increased while plasma obestatin level and intragastric pH were significantly decreased ($P4 \leq 0.01$).

Also, by comparing the changes in treatment groups at baseline, one month and six months after eradication therapy, highly significant changes between the three groups were observed with regard to all characteristics except age, sex (ANOVA 1:$p \leq 0.05$). There were marked improvement (increase of plasma and gastric ghrelin levels and ghrelin/obestatin ratio; decrease of obestatin and intragastric pH) especially after six months.

In *H. pylori*+ve group, patients are divided into mild, moderate, severe *H. pylori* density and colonization. No initial significant differences between the three subgroups were observed with

Table 6: Spearman correlations (r) in group I (*H. pylori*+ve) at baseline; significance if p≤0.05 (2-tailed)

Parameters	Gastritis scores	Ghrelin	G/O ratio	Ghrelin mRNA	pH
Gastritis score					
r	1	-0.805	-0.445	-0.714	0.59
p-value	.	0.001	0.001	0.001	0.001
Ghrelin					
r	-0.805	1	0.631	0.907	-0.493
p-value	0.001	.	0.001	0.001	0.001
Obestatin					
r	-0.48	0.579	0.326	0.485	-0.405
p-value	0.001	0.001	0.003	0.001	0.001
Ghrelin/obestatin ratio					
r	-0.445	0.631	1	0.541	-0.238
p-value	0.001	0.001	.	0.001	0.029
Ghrelin mRNA					
r	-0.714	0.907	0.541	1	-0.564
p-value	0.001	0.001	0.001	.	0.001
Intragastric pH					
r	0.59	-0.493	-0.238	-0.564	1
p-value	0.001	0.001	0.016	0.001	.
BMI					
r	-0.506	0.481	0.437	0.329	0.52
p-value	0.001	0.001	0.016	0.001	0.001

regard to age, sex and VAS (ANOVA 2:p≤0.05) but there were highly significant changes between the three subgroups with regard to other characteristics including plasma ghrelin and obestatin levels, ghrelin/obestatin ratio, gastric ghrelin mRNA expression levels, total gastritis score, MNC infiltration, BMI and intragastric pH (ANOVA 2:p≤0.05). The more *H. pylori* density and colonization in both gastric compartments especially the corpus, the more depletion in gastric ghrelin mRNA expression levels and consequently reduction in plasma ghrelin concentrations.

Table 6 shows the spearman correlations (r) in group I (*H. pylori*+ve) at baseline; significance only if p≤0.05 (2-tailed). In patients of Group I, gastric ghrelin mRNA expression as well as plasma ghrelin and obestatin levels, ghrelin/obestatin ratio and BMI were significantly negatively correlated with total gastritis scores (esp. corporal score, *H. pylori* density, PMN) (r = -0.714, p = 0.000) and intragastric pH (r = -0.564, p = 0.000). On the other hand, gastric ghrelin mRNA expression was positively correlated with plasma ghrelin and obestatin levels, ghrelin/obestatin ratio and BMI (r = 0.907, 0.485, 0.541 and 0.520; p = 0.000, 0.000, 0.000 and 0.000, respectively). As regard to intragastric pH, there was significantly positively correlated with total gastritis scores. (r = 0.590, p = 0.000).

Figure 1 shows the mean values of plasma ghrelin and obestatin levels and gastric ghrelin mRNA expression level in *H. pylori*+ve and *H. pylori*-ve subjects. Bars indicate mean values. In HP+ve group, the plasma ghrelin levels and gastric ghrelin mRNA expression level were significantly lower compared with HP-ve group while plasma obestatin level was significantly higher in *H. pylori*+ve group (p<0.001).

Figure 2 shows the mean values of plasma obestatin, gastric ghrelin mRNA expression level and total gastritis scores in *H. pylori*+ve subjects in relation to plasma ghrelin concentrations. Bars indicate mean values. *H. pylori*+ve subjects with low plasma ghrelin levels<200 pg mL^{-1} had

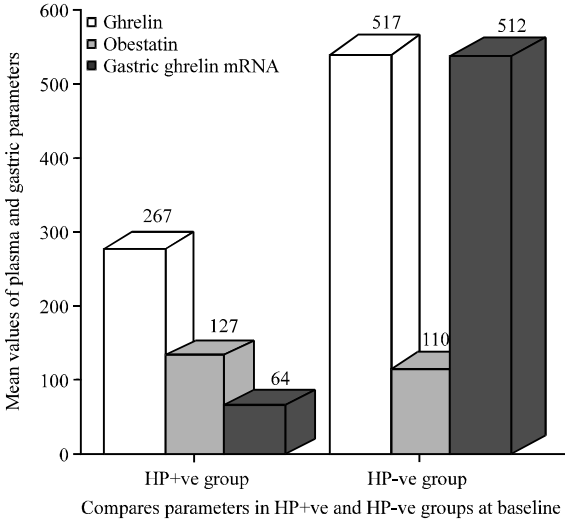

Fig. 1: Mean values of plasma ghrelin and obestatin levels and gastric ghrelin mRNA expression level in *H. pylori*+ve and *H. pylori*-ve subjects, Bars indicate mean values

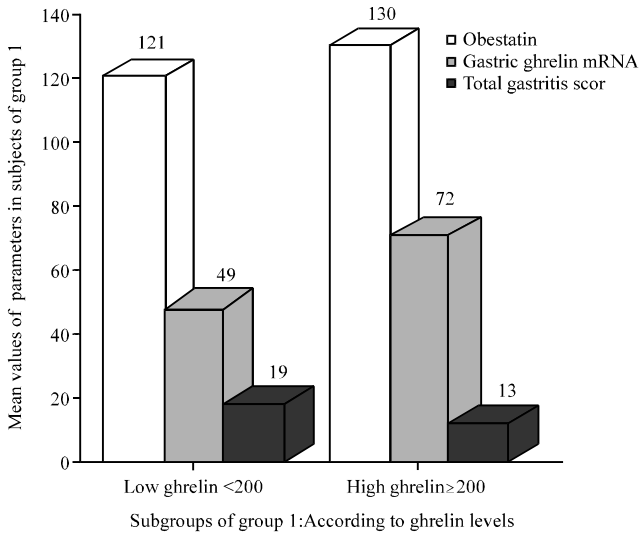

Fig. 2: Mean values of plasma obestatin, gastric ghrelin mRNA expression level and total gastritis scores in subgroups of group I (*H. pylori*+ve subjects) according to plasma ghrelin concentrations. Bars indicate mean values

significantly lower gastric ghrelin mRNA expression level, plasma obestatin level and ghrelin/obestatin ratio and significantly higher gastritis scores (total, PMN, MNC) ($p<0.001$) compared with higher ghrelin subgroup (ghr.≥200 pg mL^{-1}).

Figure 3 shows the mean values of plasma ghrelin, obestatin and gastric ghrelin mRNA expression level in *H. pylori*+ve subjects in subgroups of group I (*H. pylori*+ve subjects) according to the severity of gastritis and *H. pylori* density and colonization. Bars indicate mean values. In *H. pylori*+ve subjects, the more severity of gastritis in both gastric compartments especially the corpus (*H. pylori* density and colonization and PMN infiltrations), the more depletion in gastric

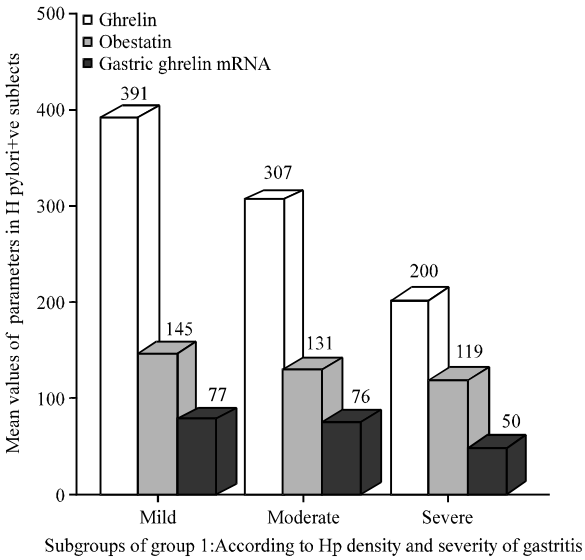

Fig. 3: Mean values of plasma ghrelin, obestatin and gastric ghrelin mRNA expression level in *H. pylori*+ve subjects in subgroups of group I (*H. pylori*+ve subjects) according to the severity of gastritis and *H. pylori* density and colonization. Bars indicate mean values

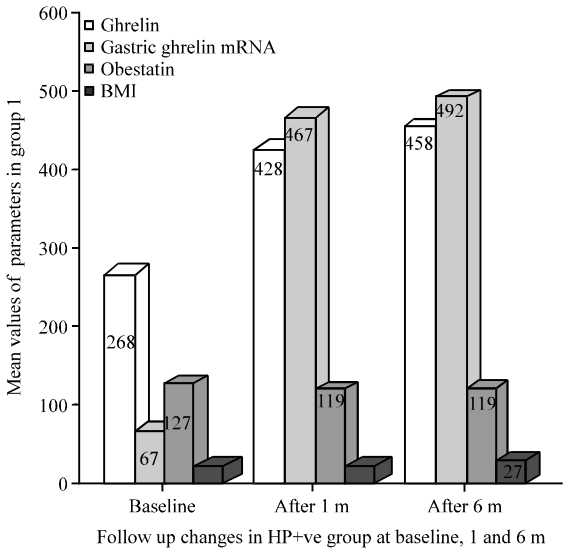

Fig. 4: Changes in plasma ghrelin, obestatin, gastric ghrelin mRNA expression levels and BMI in treatment group (group Ia) at baseline, one month and six months after eradication therapy

ghrelin mRNA expression levels and consequently reduction in plasma ghrelin concentrations and the more depletion in plasma obestatin levels (Anova 2:$p \leq 0.05$).

Figure 4 shows the changes in plasma ghrelin, obestatin, gastric ghrelin mRNA expression levels and BMI in treatment group (group Ia) at baseline, one month and six months after eradication therapy. There were significant changes in plasma and gastric ghrelin levels being increased after eradication (Anova 1; $p \leq 0.01$) BMI increased also but significant only after six months (Anova 1; $p \leq 0.01$). The more increase in gastric ghrelin mRNA expression level, the more increase in plasma ghrelin level (positive correlations) (Anova 1; $p \leq 0.01$).

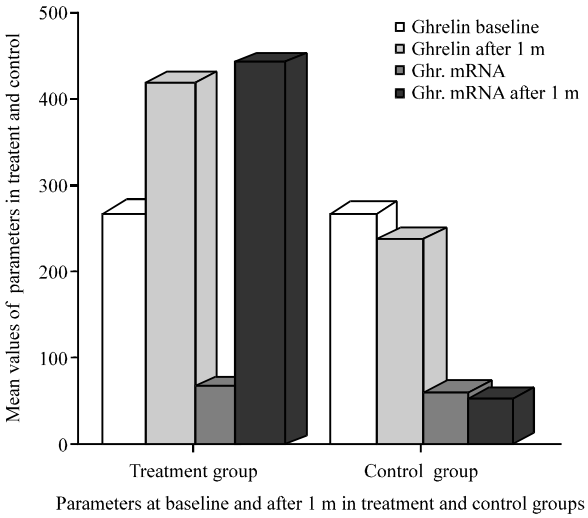

Fig. 5: Changes in plasma and gastric ghrelin mRNA expression levels in treatment and control groups at baseline and one month later after eradication therapy

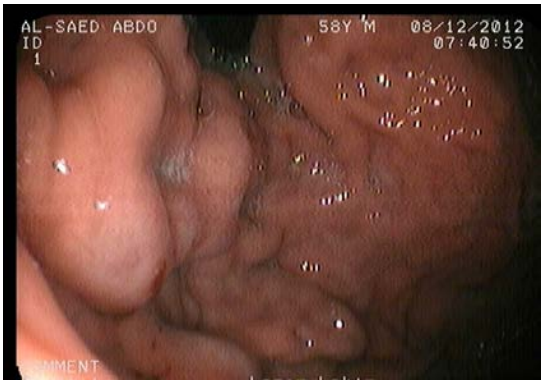

Fig. 6: Endoscopic macroscopic picture of the fundus showing gastritis in the form of hyperemia of the fundic mucosa with some superficial erosions

Figure 5 shows the changes in plasma and gastric ghrelin mRNA expression levels in treatment and control groups at baseline and one month later after eradication therapy. In treatment group, there were significant changes in plasma and gastric ghrelin levels being increased after eradication ($p4 \leq 0.01$) while in the control group, these parameters were decreased significantly one month from the baseline ($p5 \leq 0.01$).

Figure 6 shows the endoscopic macroscopic picture of the fundus showing gastritis in the form of hyperemia of the fundic mucosa with some superficial erosion.

Figure 7 gastric mucosa showing moderate number of *H. pylori* (arrows) on luminal surface of epithelial cells of gastric pits (crossed arrow). (H and E×1000).

Figure 8 gastric mucosa showing neutrophils among the rest of inflammatory cells with invasion of a gastric gland (crossed arrow) by a neutrophil (arrow). (H and E×1000).

Figure 9 gastric mucosa showing infiltration of the lamina propria of mucosa by lymphocytes (arrowheads), plasma (arrows) cells and neutrophils (crossed arrows). (H and E×1000).

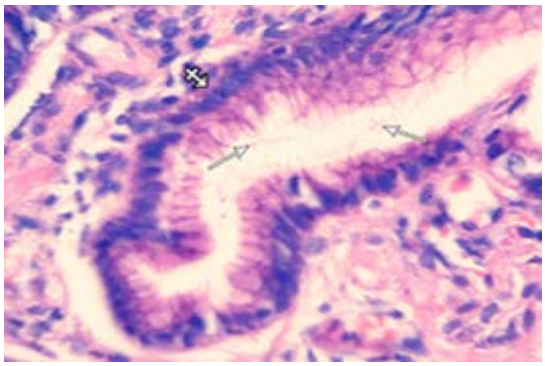

Fig. 7: Gastric mucosa showing moderate number of *H. pylori* (arrows) on luminal surface of epithelial cells of gastric pits (crossed arrow) (H and E×1000)

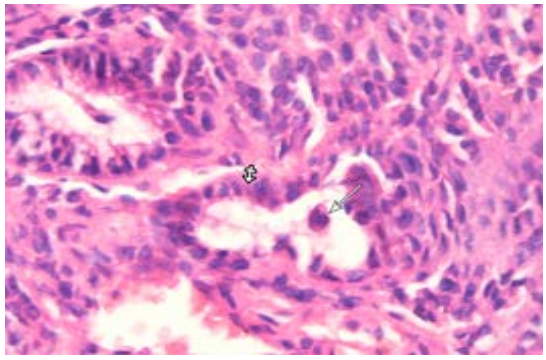

Fig. 8: Gastric mucosa showing neutrophils among the rest of inflammatory cells with invasion of a gastric gland (crossed arrow) by a neutrophil (arrow), (H and E×1000)

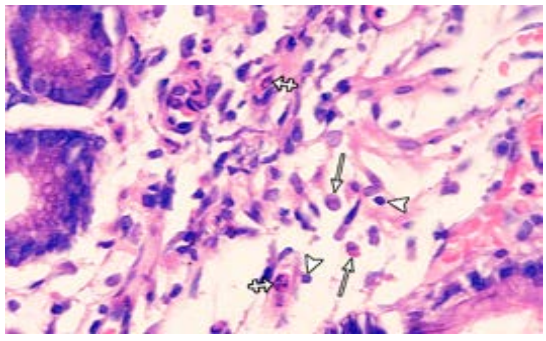

Fig. 9: Gastric mucosa showing infiltration of the lamina propria of mucosa by lymphocytes (arrowheads), plasma (arrows) cells and neutrophils (crossed arrows). (H and E×1000)

Figure 10 gastric mucosa showing infiltration of the lamina propria of mucosa by mononuclear (MNC) inflammatory cells (arrowheads) (H and E×400).

Figure 11 gastric mucosa showing infiltration of the lamina propria of mucosa by excess aggregates of inflammatory cells (arrow) (H and E×400).

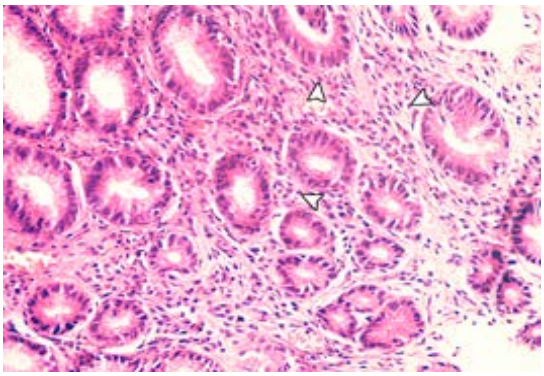

Fig. 10: Gastric mucosa showing infiltration of the lamina propria of mucosa by mononuclear inflammatory cells (arrowheads) (H and E×400)

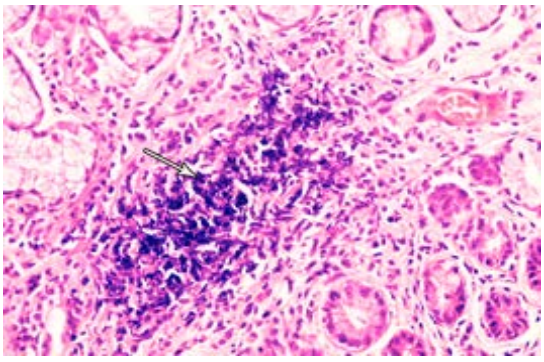

Fig. 11: Gastric mucosa showing infiltration of the lamina propria of mucosa by excess aggregates of inflammatory cells (arrow) (H and E×400)

Fig. 12: Gram's stain film of Helicobacter pylori in culture showing: Gram negative curved and spiral rods as seen with oil immersion lens (Gram's stain×1000)

Figure 12 Gram's stain film of Helicobacter pylori in culture showing: Gram negative curved and spiral rods as seen with oil immersion lens (Gram's stain×1000).

DISCUSSION

Helicobacter pylori is a Gram negative curved or S shaped rods. It has a worldwide prevalence. More than 50% of the world's populations are infected with *H. pylori*. Infection with multiple strains is quite common and occurs more frequently and with higher prevalence in developing countries (Brown, 2000). *H. pylori*, if not treated will persist throughout life. It has specificity of tissue involvement. It is exclusively inhabits mucosal cells of gastric glands. It infects and lives only in the human stomach. The enzymatic pathways needed for *H. pylori* survival in the harsh milieu of stomach are continuously switched on (Hofman *et al*., 2004).

H. pylori is a uncommonly well adapted parasite which persists indefinitely in the highly cruel environment of the stomach, despite the robust humoral and cellular immune response mounted against it. In addition, *H. pylori* is associated with specific gastroduodenal pathology and various extra-digestive diseases such as cardiovascular, immunological and a variety of other pathologies (Gasbarrini *et al*., 1999, 2004).

The assumed pathogenic determinants of *H. pylori* include virulence factors, which mediate the pathogenetic effects of the bacterium and maintenance factors, which allow the bacterium to colonize and persist indefinitely within the host. Virulence factors contribute to the three major pathogenic effects of *H. pylori*: gastric inflammation, disruption of the gastric mucosal barrier and alteration of gastric physiology (Hofman *et al*., 2004).

H. pylori is most commonly and specifically associated with chronic superficial gastritis which characterized by mononuclear inflammatory cell (MNC) infiltration associated with polymorphonuclear neutrophilic infiltration (PMN) of the epithelium. *H. pylori* is not specifically associated with metaplastia, granuloma formation, or fundic gland atrophy (Kim and Baek, 1999). *H. pylori* infection of the gastric mucosa in humans also has been shown to alter the normal gastric physiology (Del Giudice and Michetti, 2004).

Ghrelin, a 28 amino acid peptide, is an orexigenic and appetite-modulating hormone discovered by Kojima *et al*. (1999) and primarily produced in and secreted from specific enteroendocrine cells of the oxyntic mucosa located mainly in the corpus and fundus of the stomach (Kojima *et al*., 1999). Ghrelin is secreted into the extracellular matrix and then into the blood via a capillary network in the gastric lamina propria. Previous studies have demonstrated that ghrelin is involved in appetite, food intake, body-weight regulation and energy homeostasis (Bellone *et al*., 2003).

Ghrelin is also expressed in multiple tissues including small and large intestines (enteroendocrine cells distributed throughout the gut), lung, kidney, hypothalamic nucleus and pancreatic A cells (Ariyasu *et al*., 2001). It exerts endocrine, paracrine and autocrine effects. It has been demonstrated that several clinical factors including BMI, food intake and serum insulin levels have been associated with plasma ghrelin levels.

Ghrelin targets central nervous system GHS-R and exerts endocrine, paracrine and autocrine effects. Ghrelin exerts a complex interplay with other appetite-modulating peptides such as leptin, obestatin, neuropeptide Y, agouti-related protein, pro-opiomelanocortin and adiponectin, Ghrelin stimulates GHS-R present mainly in the pituitary and hypothalamic nuclei and via a GHRH-dependent mechanism, will stimulate growth hormone and insulin-like growth factor 1 (ILGF-1) release. The released hormones (GH and ILGF-1) affect a range of physiological processes. It has been suggested that ghrelin exerts a gastro-protective actions on the gastric mucosa (Slomiany and Slomiany, 2011a, b). In the alimentary tract, ghrelin increases acid secretion and stimulates gastric emptying via vagal activation (Locatelli *et al*., 2005). Ghrelin levels change significantly depending on the body's energy requirements (Tschop *et al*., 2000).

H. pylori infection is considered as an important factor in reduced appetite. Additionally, some authors reported that *H. pylori* eradication resulted in improvement in dyspeptic symptoms among patients with non-ulcer dyspepsia (Konturek *et al.*, 2006). So, it perhaps not surprising that ghrelin changes induced by *H. pylori* infection and/or eradication may also contribute to the changes of appetite or dyspeptic symptoms in those people with *H. pylori* infections.

Studies are being conducted to determine the effect of sociological and environmental factors on ghrelin release. The plasma ghrelin concentrations in gastrectomized patients still remain about one third of those in normal subjects (Cummings *et al.*, 2002). In this study, the gastric mucosa was focused on to better understand the effects of *H. pylori* infection on the alterations of ghrelin expression.

It is hypothesized that *H. pylori* infection may attenuate ghrelin production in the stomach and consequently may reduce plasma ghrelin concentrations in *H. pylori*-positive subjects. The present study was specifically designed to evaluate the impact of *H. pylori* infection and its eradication on the plasma levels and gastric mRNA expression of ghrelin, obestatin and ghrelin/obestatin ratio.

In agreement with previous studies (Osawa *et al.*, 2005), a significant association between ghrelin and *H. pylori*-associated chronic gastritis were found in current study. Plasma ghrelin concentrations and gastric ghrelin mRNA expression levels were significantly lower in *H. pylori* infection, colonization and its associated chronic gastritis than *H. pylori*-ve subjects. The presenting data also showed that in patients with *H. pylori* gastritis, eradication of the infection reversed these negative effects.

An interesting result obtained in this study was the finding that gastric ghrelin mRNA expression and circulating plasma ghrelin levels were significantly negatively correlated with density of *H. pylori* colonization and histological degree of gastritis according to PMNs, MNCs infiltrations of both corporal and antral mucosa (especially the corporal one).

These results are matched with those obtained by some authors reporting that *H. pylori* colonization has been negatively associated with circulating ghrelin levels and that *H. pylori* eradication can reverse these effects (Shiotani *et al.*, 2005). In addition, this study demonstrated that patients with histologically higher degrees of corporal gastritis in the *H. pylori*-positive subjects tend to have lower plasma ghrelin concentrations than antral gastritis.

There were no statistically significant differences between gastritis scores in *H. pylori*-negative subjects with chronic gastritis and *H. pylori*-positive subjects with chronic gastritis. However, *H. pylori*-negative subjects with chronic gastritis tend to have significantly higher plasma and gastric ghrelin concentrations, ghrelin/obestatin ratio and higher obestatin levels than *H. pylori*-positive subjects. Also, at one month follow up after *H. pylori* eradication, the plasma and gastric ghrelin concentrations was recognized to be increased significantly but with insignificant change in gastritis scores (MNC infiltrations).

These contradictory results could suggest that there is another factor rather than chronic gastritis which depleted gastric ghrelin expression and secretion and consequently reduced plasma ghrelin levels. *H. pylori* was supposed to be that factor. This may be explained as *H. pylori* induced corporal gastritis may reduce the number and/or the function of ghrelin-producing cells in the fundic mucosa. Virulence factors of *H. pylori* contribute to three major pathogenic effects:gastric inflammation, alteration of gastric physiology and disruption of the gastric mucosal barrier (Hofman *et al.*, 2004).

As all patients with atrophic body gastritis were excluded, it could be supposed that *H. pylori* infection resulted in reduction of the function of ghrelin-producing cells in the fundic mucosa

and that eradication of *H. pylori* infection resulted in rapid and considerable recovery of ghrelin-secretory function before any apparent resolution of inflammation or restoration of ghrelin producing cell numbers.

In *H. pylori* positive patients (group I), gastric ghrelin mRNA expression as well as plasma ghrelin and obestatin levels, ghrelin/obestatin ratio and BMI were significantly negatively correlated with total gastritis scores (esp. corporal score, *H. pylori* density, PMN). On the other hand, gastric ghrelin mRNA expression was positively correlated with plasma ghrelin and obestatin levels, ghrelin/obestatin ratio and BMI.

Conceding with the results obtained in this study, previous studies suggested that inflammation of the gastric mucosa is the one of the important mechanisms of *H. pylori* induced change of ghrelin production (Isomoto *et al*., 2005). In this study, that greater inflammation in an *H. pylori*-infected gastric mucosa resulted in less ghrelin production.

H. pylori-mediated gastric inflammation critically depends on the efficient recruitment and activation of macrophages, with appropriate activation of nuclear factor NF-κB (Yanai *et al*., 2008). NF-κB is a transcription factor which plays an important role in activation and expression of pro-inflammatory cytokines (TNF-α). It is known that activation of NF-κB and consequently expression of TNF-α is significantly inhibited by exogenous ghrelin administration (Konturek *et al*., 2006) thus decreasing the gastric inflammation (a gastro-protective action) (Jeffery *et al*., 2011).

Thus, it could be supposed that ghrelin exerts gastro-protective actions. In this study, greater inflammation in an *H. pylori*-infected gastric mucosa resulted in less ghrelin production thus less inhibition of proinflammatory cytokines and more inflammation and ghrelin has potent gastro-protective actions.

This assumption is not able to answer why suppression of gastric ghrelin mRNA expression does not develop in all infected subjects. However, it is possible that the presence of specific pathogenic bacterium strains could play a striking role in decreased gastric ghrelin mRNA expression. So, further studies are needed to assess the effects of pathogenic bacterial strains on gastric ghrelin mRNA expression.

Obestatin, an anorectic hormone, is a 23-amino acid peptide hormone identified in 2005. It is derived from preproghrelin, the same peptide precursor as ghrelin. In contrast to ghrelin, obestatin acts as an anorectic hormone, decreasing food intake and reducing body weight gain. The stomach is considered an important organ for obestatin secretion. Obestatin can be purified and extracted from the stomach and demonstrated to be lower in gastrectomy patients (Huda *et al*., 2008; Lacquaniti *et al*., 2011). It is also not surprising that *H. pylori* infection or its treatment can affect gastric obestatin production.

In contrary to a previous study conducted by Lee *et al*. (2010), this work could demonstrate a clear association with plasma obestatin level being increased significantly in *H. pylori* infected subjects and fell significantly after its eradication. Also, in the present study, the post-treatment plasma ghrelin/obestatin ratio significantly increased.

Eradication of Helicobacter pylori is accompanied by an array of metabolic and hormonal changes in the host. Weight gain following *H. pylori* eradication is a poorly understood phenomenon and probably results from an interaction between multiple factors. Ghrelin and obestatin are involved in the regulation of food intake and appetite and may account for some of these changes. Weight gain following *H. pylori* eradication may be attributable to changes in plasma and gastric ghrelin and obestatin (Azuma *et al*., 2002).

To investigate this hypothesis, the changes in ghrelin and obestatin production were evaluated at 4 weeks and 6 months after *H. pylori* eradication. Plasma and gastric ghrelin mRNA expression and ghrelin/obestatin ratio were found to be significantly increased, whereas plasma obestatin was decreased after H. pylori eradication. In parallel with these changes, the visual analog scales for hunger (VAS) and prospective food consumption were significantly increased after H. pylori eradication (only after 6 moths). Four weeks duration was too short to evaluate the changes in body weight and BMI after *H. pylori* eradication. In contrast, the body weight and BMI were increased significantly 6 months after *H. pylori* eradication.

CONCLUSION

The present study indicates that plasma and gastric ghrelin mRNA levels were much lower in *H. pylori*-positive patients than in *H. pylori*-negative controls using real-time quantitative RT-PCR. In contrast, plasma obestatin levels were much higher in *H. pylori*-positive patients than in *H. pylori*-negative controls. Moreover, ghrelin/obestatin ratio was in parallel with their gastric mRNA expression levels in *H. pylori*-positive patients. Therefore, the attenuation of the ghrelin production in the gastric mucosa may account for the decrease in the plasma ghrelin concentrations in *H. pylori*-positive individuals.

This study also demonstrated that alterations in gastric ghrelin mRNA expression and plasma ghrelin, obestatin and ghrelin/obestatin ratio reversed after *H. pylori* eradication. These alterations of the plasma gastric originated appetite-controlling hormones may not be due to *H. pylori* per se but may be related to the degree of gastric inflammation present.

It is the matter of corporal pathology rather than antral pathology which determines the alterations in the intragastric milieu and consequently the changes in ghrelin/obestatin balance in *H. pylori* infected subjects.

Collectively, these observations provide novel insights for understanding the physiological function of ghrelin and obestatin and their relations to various diseases. The ghrelin/obestatin balance may be a key factor which determines an individual's response to *H. pylori* infection and weight gain following *H. pylori* eradication.

RECOMMENDATIONS

These observations may be used in future research to study the appetite-modulating peptides release and to discuss the effect of selected factors on their release process. Further studies are needed to assess the effects of pathogenic bacterial strains on gastric ghrelin mRNA expression and to delineate the role of gastric ghrelin in pathological processes induced by *H. pylori*. More studies are required to explicate the determinants of plasma ghrelin levels and the complex interplay between factors determining weight gain following *H. pylori* eradication.

REFERENCES

Ariyasu, H., K. Takaya, T. Tagami, Y. Ogawa and K. Hosoda *et al.*, 2001. Stomach is a major source of circulating ghrelin and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. J. Clin. Endocrinol. Metab., 86: 4753-4758.

Asakawa, A., A. Inui, T. Kaga, H. Yuzuriha and T. Nagata *et al.*, 2001. Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. Gastroenterology, 120: 337-345.

Azuma, T., H. Suto, Y. Ito, A. Muramatsu and M. Ohtani *et al.*, 2002. Eradication of *Helicobacter pylori* infection induces an increase in body mass index. Aliment. Pharmacol. Ther., 16: 240-244.

Bartuzi, Z., E. Gawronska-Ukleja and B. Romanski, 2000. Macroscopic picture of gastric mucosa in endoscopic examination of patients with chronic gastritis and food allergy. Med. Sci. Monit., 6: 1203-1208.

Bellone, S., A. Rapa, D. Vivenza, A. Vercellotti and A. Petri *et al.*, 2003. Circulating ghrelin levels in newborns are not associated to gender, body weight and hormone parameters but depend on the type of delivery. J. Endocrinol. Invest., 26: RC9-11.

Brown, L.M., 2000. Helicobacter pylori: Epidemiology and routes of transmission. Epidemiol. Rev., 22: 283-297.

Cummings, D.E., D.S. Weigle, R.S. Frayo, P.A. Breen, M.K. Ma, E.P. Dellinger and J.Q. Purnell, 2002. Plasma ghrelin levels after dietinduced weight loss or gastric bypass surgery. N. Engl. J. Med., 346: 1623-1630.

Date, Y., M. Kojima, H. Hosoda, A. Sawaguchi and M.S. Mondal *et al.*, 2000. 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, 141: 4255-4261.

De Boer, W.A., R.J. van Etten, R.W. Schade, M.E. Ouwehand, P.M. Schneeberger and G.N. Tytgat, 1996. 4-day lansoprazole quadruple therapy: A highly effective cure for *Helicobacter pylori* infection. Am. J. Gastroenterol., 91: 1778-1782.

Del Giudice, G. and P. Michetti, 2004. Inflammation, immunity and vaccines for *Helicobacter pylori*. Helicobacter, 9: 23-28.

Dixon, M.F., R.M. Genta, J.H. Yardley and P. Correa, 1996. Classification and grading of gastritis. The updated Sydney system. Am. J. Surg. Pathol., 20: 1161-1181.

Drossman, D.A., 2006. The functional gastrointestinal disorders and the Rome III process. Gastroenterology, 130: 1377-1390.

Gasbarrini, A., E. Carloni, G. Gasbarrini and S.A. Chisholm, 2004. *Helicobacter pylori* and extragastric diseases-other *Helicobacters*. Helicobacter, 9: 57-66.

Gasbarrini, A., F. Franceschi and D.B. De Miquel, 1999. Role of *Helicobacter pylori* in extradigestive diseases. Curr. Opin. Gastroenterol., 15: 29-33.

Gokcel, A., Y. Gumurdulu, F. Kayaselcuk, E. Serin, B. Ozer, A.K. Ozsahin and N. Guvener, 2003. Helicobacter pylori has no effect on plasma ghrelin levels. Eur. J. Endocrinol., 148: 423-426.

Goodwin, C.S., M.M. Mendall and T.C. Northfield, 1997. *Helicobacter pylori* infection. Lancet, 349: 265-269.

Hofman, P., B. Waidner, V. Hofman, S. Bereswill, P. Brest and M. Kist, 2004. Pathogenesis of *Helicobacter pylori* Infection. Helicobacter, 9: 15-22.

Huda, M.S., B.H. Durham, S.P. Wong, D. Deepak and D. Kerrigan *et al.*, 2008. Plasma obestatin levels are lower in obese and post-gastrectomy subjects, but do not change in response to a meal. Int. J. Obes., 32: 129-135.

Isomoto, H., H. Ueno, Y. Nishi, C.Y. Wen, M. Nakazato and S. Kohno, 2005. Impact of *Helicobacter pylori* infection on ghrelin and various neuroendocrine hormones in plasma. World J. Gastroenterol., 11: 1644-1648.

Jeffery, P.L., M.A. McGuckin and S.K. Linden, 2011. Endocrine impact of *Helicobacter pylori*: Focus on ghrelin and ghrelin o-acyltransferase. World J. Gastroenterol., 17: 1249-1260.

Kim, D.Y. and J.Y. Baek, 1999. The comparison of histologic gastritis in patients with duodenal ulcer, chronic gastritis, gastric ulcer and gastric cancer. Yonsei Med. J., 40: 14-19.

Kim, H.B., E.S. Lee, S.W. Oh, Y.H. Kim and D.E. Lee *et al.*, 2008. Validity, reproducibility of visual analogue scales in assessment of appetite sensations. J. Korean Acad. Fam. Med., 29: 736-745.

Kojima, M., H. Hosoda, Y. Date, M. Nakazato, H. Matsuo and K. Kangawa, 1999. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature, 402: 656-660.

Konturek, P.C., T. Brzozowski, B. Walter, G. Burnat, T. Hess, E.G. Hahn and S.J. Konturek, 2006. Ghrelin-induced gastroprotection against ischemia-reperfusion injury involves an activation of sensory afferent nerves and hyperemia mediated by nitric oxide. Eur. J. Pharmacol., 536: 171-181.

Lacquaniti, A., V. Donato, V. Chirico, A. Buemi and M. Buemi, 2011. Obestatin: An interesting but controversial gut hormone. Ann. Nutr. Metab., 59: 193-199.

Lee, E.S., Y.S. Yoon, C.Y. Park, H.S. Kim, T.H. Um, H.W. Baik and E.J. Jang, 2010. Eradication of *Helicobacter pylori* increases ghrelin mRNA expression in the gastric Mucosa. J. Korean Med. Sci., 25: 265-271.

Locatelli, V., E. Bresciani, I. Bulgarelli, D. Rapetti and A. Torsello *et al.*, 2005. Ghrelin in gastroenteric pathophysiology. J. Endocrinol. Invest., 28: 843-848.

Mackey, K. and P. Chomczynski, 1996. Long-term stability of RNA isolation reagents. J. NIH Res., 8: 72-78.

Masuda, Y., T. Tanaka, N. Inomata, N. Ohnuma and S. Tanaka *et al.*, 2000. Ghrelin stimulates gastric acid secretion and motility in rats. Biochem. Biophys. Res. Commun., 276: 905-908.

Nakazato, M., N. Murakami, Y. Date, M. Kojima, H. Matsuo, K. Kangawa and S. Matsukura, 2001. A role for ghrelin in the central regulation of feeding. Nature, 409: 194-198.

Nweneka, C.V. and A.M. Prentice, 2011. *Helicobacter pylori* infection and circulating ghrelin levels: A systematic review. BMC Gastroenterol., Vol. 11, 10.1186/1471-230X-11-7

Nwokolo, C.U., D.A. Freshwater, P. O'Hare and H.S. Randeva, 2003. Plasma ghrelin following cure of *Helicobacter pylori*. Gut, 52: 637-640.

Osawa, H., 2008. Ghrelin and *Helicobacter pylori* infection. World J. Gastroenterol., 14: 6327-6333.

Osawa, H., M. Nakazato, Y. Date, H. Kita and H. Ohnishi *et al.*, 2005. Impaired production of gastric ghrelin in chronic gastritis associated with *Helicobacter pylori*. J. Clin. Endocrinol. Metab., 90: 10-16.

Patrono, C. and B.A. Peskar, 1987. Radioimmunoassay in Basic and Clinical Pharmacology (Handbook of Experimental Pharmacology). Springer-Verlag, Berlin, Germany.

Schreiber, S., M. Konradt, C. Groll, P. Scheid and G. Hanauer *et al.*, 2004. The spatial orientation of *Helicobacter pylori* in the gastric mucus. Proc. Natl. Acad. Sci. USA., 101: 5024-5029.

Shiotani, A., T. Miyanishi, N. Uedo and H. Iishi, 2005. Helicobacter pylori infection is associated with reduced circulating ghrelin levels independent of body mass index. Helicobacter, 10: 373-378.

Slomiany, B.L. and A. Slomiany, 2011a. *Helicobacter pylori* induces disturbances in gastric Mucosal Akt activation through inducible nitric oxide Synthase-dependent S-nitrosylation: Effect of Ghrelin. Gastroenterology, 10.5402/2011/308727

Slomiany, B.L. and A. Slomiany, 2011b. Ghrelin suppression of *Helicobacter pylori*-induced S-nitrosylation-dependent Akt inactivation exerts modulatory influence on gastric mucin synthesis. Inflammopharmacology, 19: 89-97.

Stemmermann, G.N., 1994. Intestinal metaplasia of the stomach: A status report. Cancer, 74: 556-564.

Stevens, A. and R.J. Francis, 1996. Micro Organisms. In: Theory and Practice of Histological Techniques, Bancroft, J.D., A. Stevens and D.R. Turner (Eds.). 4th Edn., Chirchill and Livingstone, New York, pp: 291-308.

Tschop, M., D.L. Smiley and M.L. Heiman, 2000. Ghrelin induces adiposity in rodents. Nature, 407: 908-913.

Uemura, N., S. Okamoto, S. Yamamoto, N. Matsumura and S. Yamaguchi *et al.*, 2001. Helicobacter pylori infection and the development of gastric cancer. N. Engl. J. Med., 345: 784-789.

Weigle, D.S., D.E. Cummings, P.D. Newby, P.A. Breen and R.S. Frayo *et al.*, 2003. Roles of leptin and ghrelin in the loss of body weight caused by a low fat, high carbohydrate diet. J. Clin. Endocrinol. Metab., 88: 1577-1586.

Yanai, A., S. Maeda, W. Shibata, Y. Hikiba and K. Sakamoto *et al.*, 2008. Activation of IkappaB kinase and NF-kappaB is essential for *Helicobacter pylori*-induced chronic gastritis in *Mongolian gerbils*. Infect. Immun., 76: 781-787.