Influence of Helicobacter Pylori Infection on Plasma Ghrelin Level in Patients with Upper GIT Lesions

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Abstract

**Background:** The stomach is the main source of circulating ghrelin. Gastric colonization by H. pylori showed to affect ghrelin dynamics. This fact is due to long-term persistent H. pylori infections leading to atrophic gastritis. Some authors reported that; the relation between H. pylori status and ghrelin level is uncertain.

**Aim of Work:** This study evaluates the effect of H. pylori infection on gastric ghrelin production and consequently the plasma ghrelin concentration.

**Subjects and Methods:** Forty patients complaining of abdominal pain, dyspepsia, acid regurge and burning pain were included in the study after giving informed consent. Estimation of ghrelin hormone level and H. pylori IgG antibody was done. The detection of ghrelin level was based on competitive enzyme immunoassay. While the detection of H. pylori was performed using quantitative estimation of H. pylori IgG, in addition to endoscopic biopsies from antrum and corpus of the stomach. All specimens were subjected to histopathological examination using hematoxylin and eosin stain to detect H. pylori.

**Results:** Ghrelin level is higher among subjects with H. pylori positive than H. pylori negative regarding the mean and individual data persons. With median in H. pylori negative (n=8) is 173.5 (44.95-589.25). Median in H. pylori positive (n=32) is 614 (253-928.38) \( p \)-value=0.052, borderline significant. As regards H. pylori; it was detected pathologically with hematoxylin and Eosin and serologically by IgG antibody detection. The pathological specimen showed that; out of 40 patients 8 (20%) were negative, while 32 patients (80%) were positive for H. pylori. On the other hand by H. pylori IgG detection on the same patients 8 patients were below 15 (non-reactive) i.e. –ve, meanwhile the remaining 32 were above 15 (reactive) i.e. +ve. So there is significant association between the two methods for detection of H. pylori \( p<0.001 \).

**Conclusion:** Marked discrepancies do exist in the result about ghrelin plasma level and H. pylori colonization has been associated with high, equivalent or low level of plasma ghrelin. So the relation between H. pylori status and ghrelin levels is uncertain. Regarding the diagnosis of H. pylori infection, it was concluded that sensitivity of H. pylori IgG antibodies versus histopathological examination was 100% and specificity was 100%, since the result was the same in diagnosis.

**Key Words:** H. pylori – H. pylori IgG antibodies – Ghrelin – Upper abdominal pain.

Introduction

GHRELIN is a 28-amino acid peptide hormone, originally identified in rat stomach as an endogenous ligand of the growth hormone secretagogue receptor (GHS-R) [1]. It is primarily secreted from X/A-like endocrine cells of the oxyntic gland-containing portion of the stomach, but is also expressed in a wide variety of other tissues including pituitary, hypothalamus, colon, kidney, placenta, ovary and testes [2].

Ghrelin administration strongly stimulates feeding, and leads to increase in body weight [3]. The stomach is the main source of circulating ghrelin and there is influence of Helicobacter pylori infection on circulating ghrelin levels [4].

Also a persistent colonization of H. pylori is associated with acute gastritis, peptic ulcer diseases and gastric adenocarcinoma. H. pylori-induced inflammatory response affects many gastric cell types, including those responsible for leptin and ghrelin production [5]. Elimination of H. pylori after anti-microbial therapy has been reported to increase nutrition and weight suggesting that H. pylori could play a role in the regulation of leptin and ghrelin expression [6].

So the aim of this study is to find out; whether H. pylori infection affects gastric ghrelin production and consequently alters plasma ghrelin concentration or not.
Patients and Methods

This study was conducted on 40 patients complaining of abdominal pain, dyspepsia, acid regurge and heart burn. These patients were recruited from endoscopy unit of Kasr El-Aini Internal Medicine Hospital. The research was performed between November 2008-August 2009.

All patients were subjected to:
A- History taking to exclude the following:
   Age not less than 18 or more than 80 years, Pregnancy, Diabetes mellitus, Systemic infection, Thyroid and liver disease, Renal impairment, Use of medications effective against Helicobacter pylori bacteria during the last 3 months, Alcohol use, Drug addiction, NSAIDS use, Gastric surgery or Cachetic state.

B- The selected cases were subjected to the following:
   - Three Blood samples were withdrawn from these patients after 12 hours fasting:
     • Plasma samples on EDTA with Aprotinin for the estimation of ghrelin hormone level.
     • Serum sample for estimation of: H. pylori IgG, liver functions (AST, ALT, Albumin), kidney functions (Creatinine), thyroid profile (TSH), lipid profile (Cholesterol, Triglycerides).
     • Plasma sample on fluoride for estimation of fasting blood sugar.
   - Weight and height were also estimated to determine body mass index (weight/height 2).
   - Endoscopic biopsies were taken from antrum and corpus of stomach and were subjected to histopathological examination using haematoxlin and eosin stain for detection of H. pylori.


Statistical analysis:
The data was coded and entered using the statistical package SPSS version 15. Parametric quantitative data were expressed as mean ± SD and compared using student t-test. Non parametric quantitative data expressed as median (percentiles) and compared using Mann whitney test. Qualitative data were expressed as frequency and percentage. Association between qualitative variables was done using correlation coefficient (r). p-values less than or equal to 0.05 were considered statistically significant.

Results

The study population included 28/40 were males (70%) and 12/40 were females (30%), aged between 19 and 58 years with a mean ± SD of 37.33±11.23.

Table (1): Frequency distribution of Body mass index (BMI) groups.

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Under-weight</td>
<td>2</td>
</tr>
<tr>
<td>Normal weight</td>
<td>14</td>
</tr>
<tr>
<td>Over-weight</td>
<td>15</td>
</tr>
<tr>
<td>Obese</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
</tr>
</tbody>
</table>

According to the body mass index (BMI) of the selected patients; where BMI up to 25 is normal, 25-30 is overweight and >30 is obese, it was found that out of the forty patients two were under weight (5%), 14 have normal body weight (35%), 15 were over weight (37.5%) and 9 were obese (22.5%).

Table (2): Association between H. pylori IgG * Hx & Eosin stain.

<table>
<thead>
<tr>
<th>Hx &amp; Eosin</th>
<th>Negative</th>
<th>Positive</th>
<th>Total</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. pylori &lt;15 count</td>
<td>8</td>
<td>0</td>
<td>8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>15 and more</td>
<td>0</td>
<td>32</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>32</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>

As regards H. pylori; it was detected pathologically with Hx and Eosin and serologically by IgG antibody detection. The pathological specimen showed that; out of 40 specimens 8 (20%) were negative, while 32 specimens (80%) were positive for H. pylori. On the other hand by H. pylori IgG detection on the same patients 8 patients were below 15 (non-reactive) i.e. –ve, meanwhile the remaining 32 were above 15 (reactive) i.e. +ve. So there is significant association between the two methods for detection of H. pylori p<0.001.

Table (3): Comparison between H. pylori positive and negative groups as regards age, BMI, ghrelin level.

<table>
<thead>
<tr>
<th>H. pylori</th>
<th>+ve</th>
<th>–ve</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>39.50±11.31</td>
<td>28.63±5.37</td>
<td>0.012*</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>26.81±4.79</td>
<td>25.21±8.57</td>
<td>0.481+</td>
</tr>
<tr>
<td>Ghrelin</td>
<td>614</td>
<td>173.5</td>
<td>0.052</td>
</tr>
<tr>
<td>(253-928.4)</td>
<td>(44.95-589.25)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Comparison between H. pylori positive and negative groups showed significantly higher age(y) in the positive group (39.50 ± 11.31) than negative group (28.63 ± 5.37), p-value (0.012), BMI showed no observed difference between negative group (25.21 ± 8.57) and positive group (26.81 ± 4.79), p-value (0.481), while ghrelin level it was significantly higher in positive group [614 (253-928.4)] than negative group [173.5 (44.95-589.25), p-value =0.052 (significant) Fig. (1)].

Table (4): Association between H. pylori* and BMI groups.

<table>
<thead>
<tr>
<th>H. pylori</th>
<th>Normal weight</th>
<th>Overweight &amp; obesity</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>7 (43.8%)</td>
<td>1 (4.2%)</td>
<td>&lt;0.004</td>
</tr>
<tr>
<td>Positive</td>
<td>9 (56.3%)</td>
<td>23 (95.8%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>24</td>
<td></td>
</tr>
</tbody>
</table>

While there was no significant difference in BMI between H.pylori positive and negative groups. Overweight and obese groups showed higher frequency of positive H. pylori 23/24 (95.8 %) than normal weight groups 9/16 (56.3%) p-value <0.004 (Fig. 2).

Table (5): Correlations between ghrelin level with age and BMI.

<table>
<thead>
<tr>
<th></th>
<th>Pearson correlation (r)</th>
<th>p-value</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.146</td>
<td>0.370</td>
<td>40</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.233</td>
<td>0.148</td>
<td>40</td>
</tr>
</tbody>
</table>

Correlation between age and BMI with ghrelin level, it was found that; ghrelin level showed insignificant +ve correlation with age (p-value =0.370) and insignificant negative correlation with BMI (p-value=0.148).

Table (6): Comparison between normal weight group, overweight and obese group as regards ghrelin level.

<table>
<thead>
<tr>
<th>Ghrelin</th>
<th>Normal weight (N=16)</th>
<th>Overweight &amp; obese (N=24)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>564.50</td>
<td>(165.25-938.50)</td>
<td>507 (188.75-921)</td>
<td>0.984</td>
</tr>
</tbody>
</table>

It was found that out of the forty patients, the median ghrelin level in the 16 patients with normal weight was 564.50 (165.25-938.50). Meanwhile it was 507.00 in the remaining 24 overweight and obese patients (188.75-921.00) with no statistical significant difference (p=0.984).

Table (7): Correlation between ghrelin levels with H. pylori.

<table>
<thead>
<tr>
<th>H. pylori</th>
<th>H. pylori p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>173.5 (44.95-589.25)</td>
</tr>
</tbody>
</table>

Ghrelin level is higher among subjects with H. pylori positive than H. pylori negative regarding the mean and individual data persons. With median in H. pylori negative (n=8) is 173.5 (44.95-589.25). Median in H. pylori positive (n=32) is 614 (253-928.38) p-value= 0.052, borderline significant (Fig. 3).
Discussion

The stomach is the main source of circulating ghrelin and there is influence of Helicobacter pylori infection on circulating ghrelin levels [4].

Also a persistent colonization of H. pylori is associated with acute gastritis, peptic ulcer diseases and gastric adenocarcinoma. H. pylori-induced inflammatory response affects many gastric cell types, including those responsible for leptin and ghrelin production [5]. Elimination of H. pylori after anti-microbial therapy has been reported to increase nutrition and weight suggesting that H. pylori could play a role in the regulation of leptin and ghrelin expression [6].

Some authors reported that; the relation between H. pylori status and Ghrelin level is uncertain [7,8].

So the aim of this study is to find out; whether H. pylori infection affects gastric ghrelin production and consequently alters plasma ghrelin concentration or not.

As regards comparison between H. pylori groups and age, we found that H. pylori (+)ve groups showed significantly higher age 39.50±11.31 than H. pylori (–)ve group 28.63±5.37, p-value =0.012. A result that is not in agreement with Gockel et al. [9] who reported that: The age was similar between the two groups of H. pylori positive and negative. Also, Jatin Roper et al. [10] reported that: No significant difference in age in the group of H. pylori positive and H. pylori negative.

Correlation between H. pylori groups and BMI was performed with a result that; there is insignificant difference of BMI in H. pylori groups. While a significant difference of H. pylori in different BMI groups was noted.

This was in agreement with the results of many studies performed by Akiko Shiotani [11] and Hajime Isomoto et al. [4] who found that: No significant difference in BMI between those with and without H. pylori infection.

These results was explained by Nishi et al. [12] and Osawa et al. [13] who reported that: One of the possible mechanism to explain these results could be the variation in leptin and ghrelin which are secondary to chronic H. pylori gastritis that in case of H. pylori infection there is enhanced production of gastric leptin and decreased production of gastric ghrelin. The latter stimulates growth hormone and has orexigenic and adipogenic effects. It does not only accelerate food passage and motility but also affect appetite and weight control [11].

Correlation between age and BMI with ghrelin plasma level was performed in this study. By statistical analysis, the results revealed that; non significant positive correlation between plasma ghrelin level and age and non-significant negative correlation with BMI among the studied subjects. Also the mean ghrelin level in the sixteen subjects with normal weight was more or less similar to the remaining twenty-four subjects, who were overweight and obese i.e. the mean ghrelin level is nearly equal among both normal weight 564.50 (165.25-938.50) and over weight subjects 507 (188.75-921) (with no statistical significant difference, p-value=0.984).

Similarly many authors [4,14,15] found that there was very weak or non significant correlation between BMI and ghrelin level. They suggested that at a population level, there are other and more important regulatory factors of growth hormone than ghrelin.

Also Shiiya et al. [16] and Haqq et al. [17] found that in adults, plasma ghrelin concentrations were lower in obese subjects, compared with those with normal body weight.

On the contrary; other studies revealed that ghrelin increases food intake and eating and leads to increased body mass index [18]. Also in a research done by Mehmet Cindoruk [19], he found that the mean fasting serum ghrelin level was higher in patients with BMI >25Kg/m² than patients with BMI <25Kg/m².

In this study the correlation of ghrelin with H. pylori groups revealed that a borderline significant difference (p-value=0.052) was noted between ghrelin level and both H. pylori positive and H. pylori negative groups. H. pylori +ve group had significantly higher ghrelin level 614 (253-928.4) than H. pylori –ve group 173.5 (44.95-589.25).

Many studies reported that the un-expected high values of circulating ghrelin in H. pylori-infected patients are due to augmented ghrelin production in the stomach, it is suggested that there may be a compensatory increase in plasma ghrelin concentration in response to the decreased density of ghrelin-producing cells. However, long-term infection by this organism leads to further extension of mucosal atrophy towards the corpus [5,20].

On the other hand, Hiroyki Osawa [21] reported that plasma ghrelin concentrations were significantly lower in H. pylori positive subjects than in H. pylori-negative control. A similar difference was also significantly observed between patients
with chronic gastritis alone and H. pylori-negative control. These results suggest that the attenuation of the ghrelin production in the gastric mucosa account for the decrease in the plasma ghrelin concentration in H. pylori positive individuals.

Also, another study was reported by Akiko Shiotani et al. [11]. They studied the relation between H. pylori infection and ghrelin level on 801 patients. They reported that the serum level of ghrelin in H. pylori positive group was lower compared to uninfected subjects. And this difference was highly significantly independent of sex and BMI, and probably regardless of gastric atrophy in young adults.

Konturek et al. [22] reported that H. pylori infection leads to a decrease of circulating ghrelin through a reduction of ghrelin-producing cells in the gastric mucosa and increases the amount of gastric leptin with no effect on circulating leptin levels. Eradication of H. pylori reverses the abnormal regulation of gastric hormone secretion. This finding is suggested to favor weight gain after H. pylori eradication and points to the potential effect of H. pylori in the pathophysiology of obesity.

During earlier stages of H. pylori colonization Nwokolo et al. [6] reported that plasma ghrelin concentrations increased after the eradication of H. pylori. Also, eradication of Helicobacter pylori Increases ghrelin mRNA Expression in the Gastric Mucosa [23].

However, still there are conflicting data, since Gokcel et al. [9], studied a small group of 39 age- and BMI-matched H. pylori-positive (n=24) and negative (n=15) women and reported that the mean ghrelin levels was similar in H. pylori infected and un-infected individuals (370.9±27 pmol/l). The authors reported that many factors should be considered regarding the other contradictory reports; (1) the difference in study populations of diverse races, nutrient status, dietary habits; (2) small sample size (3) inadequate assessment of H. pylori status, strain and virulence (4) difference in RIA protocols for ghrelin. Also, Nwokolo et al. [6] did not observe any significant post-cure rise in fasting plasma ghrelin levels following the treatment period.

Still the findings are in keeping with previous reports by Suzuki et al. [24] and Tatsuguchi et al. [14]. They also found no association between plasma Ghrelin level and H. pylori. They reported that at a population level, there are other and more important regulations factors of GH than ghrelin.

Many authors reported that H. pylori colonization has been associated with high, equivalent or low level of plasma ghrelin. So the relation between H. pylori status and Ghrelin levels is uncertain [8,22].

In conclusion, these observations provide novel insights for understanding the physiological function of ghrelin and its relation to various diseases. But still the relation between H. pylori status and plasma level of ghrelin is uncertain. So further investigations are necessary to determine different strains of H. pylori which were proved to be an important point in determination of gastric atrophy and subsequently plasma concentration of ghrelin or the more active form: Acylated ghrelin. Also, studying the secretory activity of gastric ghrelin cells is a very important factor in estimation of plasma ghrelin level.

References


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