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Study of Ghrelin and Leptin levels in Helicobacter pylori patients

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ABSTRACT

Ghrelin and leptin are important peptides that regulate food intake and body weight. Since ghrelin is secreted mainly from the acid-secreting gastric mucosa, Helicobacter pylori infection can reduce its plasma concentrations, but it is not yet certain whether gastritis caused by Helicobacter is able to alter levels of ghrelin. Leptin is mainly excreted from adipose tissues and is excreted in less quantities by the gastric mucosa. Hence, gastritis caused by H. pylori is likely to affect its plasma levels. Therefore, the study aimed to investigate the plasma levels of ghrelin and leptin in patients with Helicobacter pylori and compare them with their levels in healthy people, and determine the correlation of ghrelin and leptin with body mass index (BMI) and determine the correlation of ghrelin with leptin in the sick and healthy people. Ghrelin and leptin were assayed in 66 patients with H. pylori and 22 controls, using specific enzyme-linked immunosorbent assays (ELISA). The results showed that patients with H. pylori had lower ghrelin concentrations (803.951 ± 89.879) compared to controls (893.047 ± 80.304) while no difference in leptin concentrations was shown in patients (6.515 ± 1.817) compared to controls (5.720 ± 1.603). Ancorrelation was found between leptin and body mass index (BMI), but no correlation was observed between ghrelin and body mass index (BMI). A correlation was found between levels of ghrelin and leptin in patients with Helicobacter pylori.

Keywords: H. Pylori,Helicobacter pylori, Ghrelin, Leptin, Body Mass Index.

INTRODUCTION

Helicobacter pylori infection is the main causative factor of chronic active gastritis that lasts for years with a low healing rate, where the body picks up the infection in childhood years often under unsuitable environmental conditions. The unsuitable environment is one of the most important risk factors leading to the infection (1).

Ghrelin, a 48-amino acid peptide, has a growth hormone-releasing efficacy and plays central and peripheral roles related to food intake, gastric motility, and acid secretion(2).

Most ghrelin is produced from parietal cells in the acid-secreting gastric mucosa. Plasma concentrations of ghrelin rise before a meal and decrease after ingestion. As ghrelin affects the mass of adipose tissue, it can be considered an appetite stimulating hormone in the short-term, as this effect ends with eating, and it can also be considered a hormone that regulates body weight in the long-term (3).

Leptin is the protein product of the obesity gene, which is excreted by adipocytes. In addition, leptin is excreted in the lower half of the gastric floor glands. Leptin provides information to the central nervous system through the feedback mechanism of the size of energy stores and thus plays a role in controlling food intake, energy expenditure and body weight stability (4).

The actions of ghrelin and leptin are mediated by neurons in the arcuate nucleus in the hypothalamus containing appetite-stimulating peptides such as (neuropeptide Y) and others containing appetite-suppressing peptides. Leptin inhibits neurons of appetite-stimulating peptides while ghrelin activates neurons of appetite-stimulating peptides (5). Women have higher levels of ghrelin and leptin than men (6). Therefore, there is a possibility that chronic damage to the gastric mucosa, such as chronic gastritis caused by Helicobacter pylori, may affect the production of ghrelin and leptin from the gastric mucosa and thus the plasma concentrations of both hormones leading to a change in food intake and body weight. The study aimed to find out whether chronic H. pylori gastritis was related to the levels of plasma ghrelin and leptin and to investigate whether there was a significant gender difference in the levels of these two hormones in response to H. pylori infection.

Materials and methods: the study was conducted on 66 patient samples (39 men and 27 women) and a control group (12 men and 10 women), whose ages ranged between 20 and 60 years, from Al-Assad University Hospitals and Al-Mouwasat University Hospitals during 4 months from July 1 to October 31, 2011. The study included patients with a body mass index within the normal range between 18-25 Kg/m2, and patients with one of the following conditions were excluded: diabetes, systemic infection, cancer, liver and thyroid diseases, renal impairment, chronic use of non-steroidal anti-inflammatory drugs (NSAIDs), patients who used any of the effective medicines against H.pylori during the previous three months and all patients previously undergoing gastrointestinal surgery.

Samples were collected in the gastro-intestinal division of the upper gastrointestinal endoscopy department, where patients underwent full endoscopy of the esophagus, stomach and duodenum, and gastric biopsies were taken from the body of the stomach when there were clinical signs of chronic inflammation. Patients with moderate to severe infection with Helicobacter pylori were selected. The infection was confirmed by performing a rapid urease assay, which depends on the ability of Helicobacter pylori to convert urea to ammonia and convert the medium from acidic to alkaline, thus changing the indicator color from yellow to red. The test sensitivity is up to 90%. After confirming the infection's positivity, blood samples were collected on a dry tube and a tube containing the EDTA anticoagulant. The control samples were collected in the same way, after making sure of the absence of the infection from the negative rapid test.

The body mass index (BMI) has been assigned for patients by dividing the body weight in kilograms by the square of their height in meters.BMI = Weight (Kg) / (Length in meter)^2

Ghrelin and leptin were assayed by the Enzyme-Linked Immunosorbent Assay method with two kits from the German DRG company at Damascus University Center for Blood Transfusion. Insulin assay was carried out by means of Enzyme Linked Immunosorbent Assay (ELISA) with a kit from Source AID at Damascus University Center for Blood Transfusion. The antibody assay was carried out using the Enzyme Linked Immunosorbent Assay (ELISA) method with a kit from the German company Euroimmun at Damascus University Center for Blood Transfusion.

RESULTS

Determination and comparison of ghrelin values for patients with H.pylori and normal control groups
The mean value ± standard deviation of ghrelin concentrations in patients with Helicobacter pylori was 803.951 ± 89.879 pg / ml, and the value of this average for the control group was 893.047 ± 80.304 pg / ml.

By applying the T-Student test, a statistically significant difference (p = 0.000008) was observed between the mean values of the two groups (Figure 1)

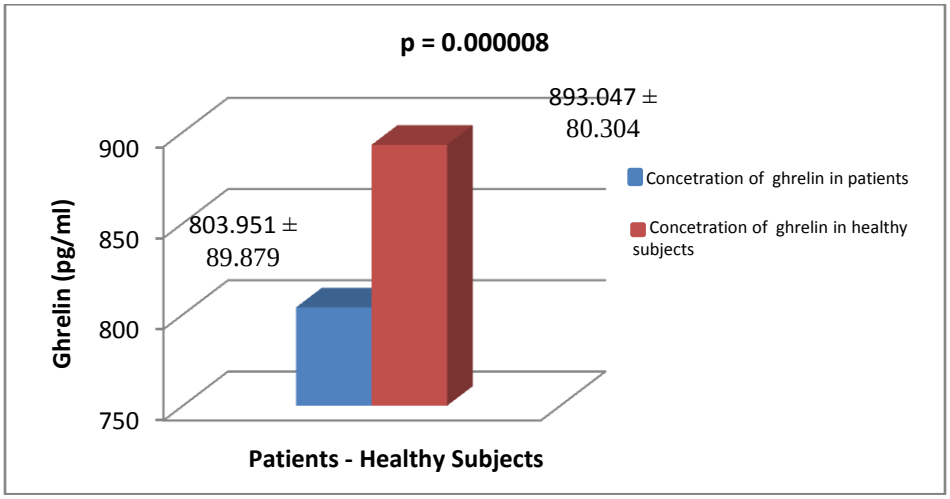

Figure 1: Comparison of ghrelin levels between H. pylori and control subjects.

Determination and comparison of leptin values in patients with Helicobacter pylori and normal control groups
The mean value ± standard deviation of leptin concentrations in patients with Helicobacter pylori was 6.515 ± 1.817 ng / ml, and the mean value for the control group was 5.720 ± 1.603 ng / ml.

By applying the T-Student test, no statistically significant difference (p = 0.058582) was observed between the mean values of the two groups (Figure 2).

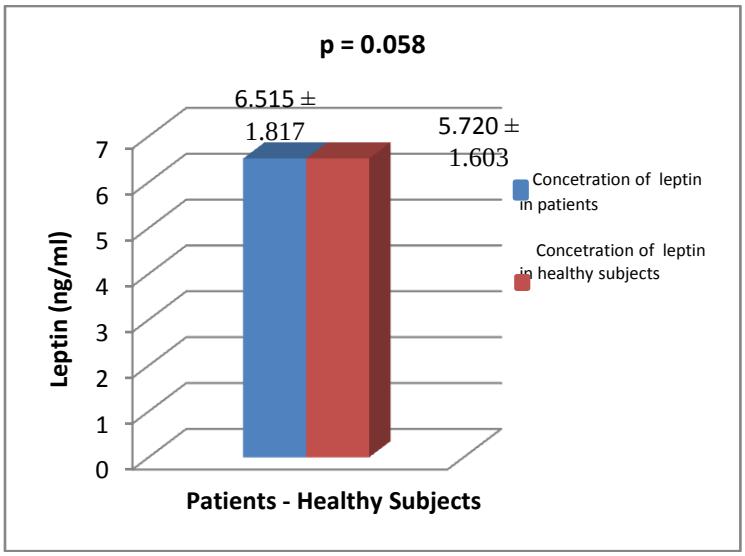

Figure 2: Comparison of leptin levels between H. pylori patients and healthy control subjects.

Determination and comparison of ghrelin values for infected and healthy men

The mean value ± standard deviation of ghrelin concentrations in infected men was 773.563 ± 77.903 pg / ml, and the value of this average in healthy men was 868.8015 ± 83.715 pg / ml.

By applying the T-Student test, a statistically significant difference (P = 0.00267) was observed between the mean values of the two groups (Figure 3).

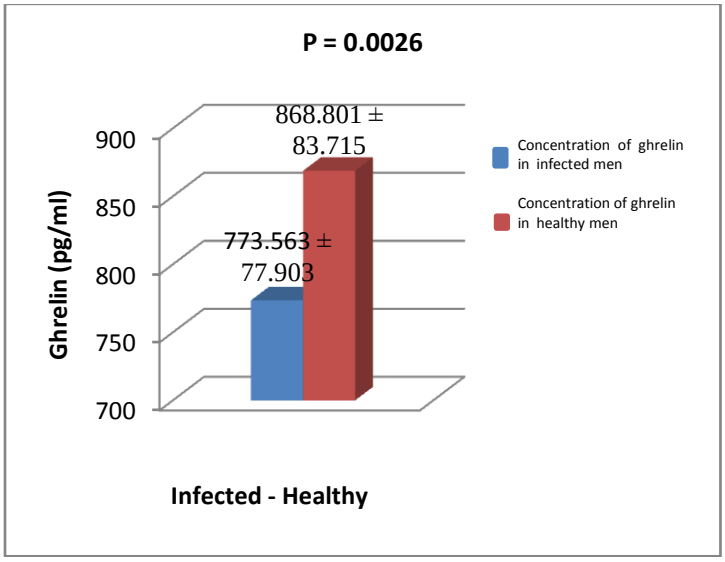

Figure 3: Comparison of ghrelin levels in infected and healthy men.

Determination and comparison of ghrelin values for infected and the healty women

The mean value ± standard deviation of ghrelin concentrations in the infected women was 847.8467 ± 89.105 Pg/ml. As for healthy women, the value of this mean was 922.1424 ± 68.971 Pg/ml.

By applying theT-Student test, no statistically significant differences were observed between the mean values of these two groups (p = 0.057792) (Figure 4).

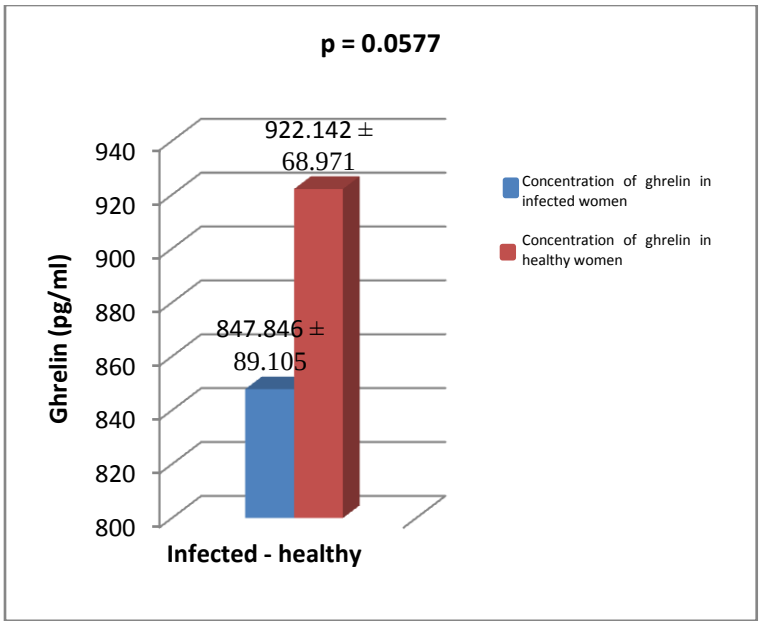

Figure 4: Comparison of ghrelin levels in infected and healthy women.

Determination and comparison of ghrelin values for infected men and infected women

The mean value ± standard deviation of ghrelin concentrations in infected men was 773.563 ± 77.903 Pg/ml, and for the infected women, this average value was 847.846 ± 89.105 Pg/ml.

By applying theT-Student test, no statistically significant differences were observed between the mean values of these two groups (p = 0.058582) (Figure 5).

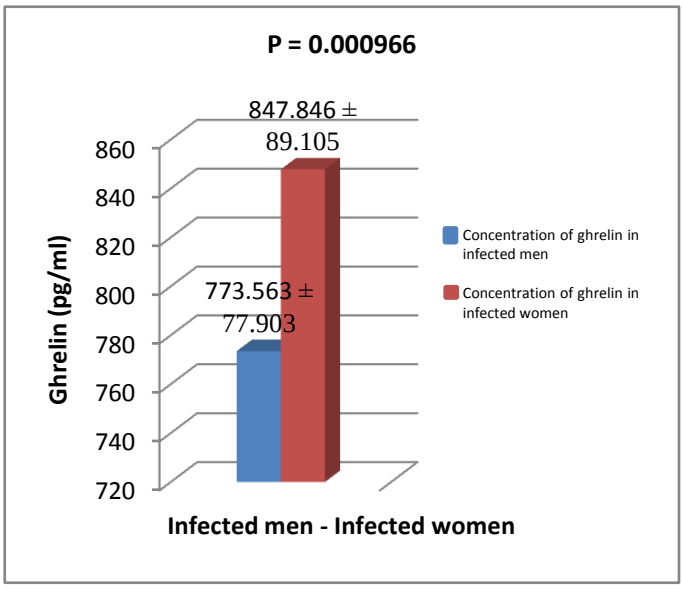

Figure 5: Comparison of ghrelin levels in infected men and infected women.

Determination and comparison of leptin values for infected men and healthy men

The mean value ± standard deviation of leptin concentrations in infected men was 5.647 ± 1.218 Ng/ml, and in the group of healthy men, the average value was 4.933 ± 1.011 Ng/ml.

By applying theT-Student test, no statistically significant differences were observed between the mean values of these two groups (p = 0.0542) (Figure6).

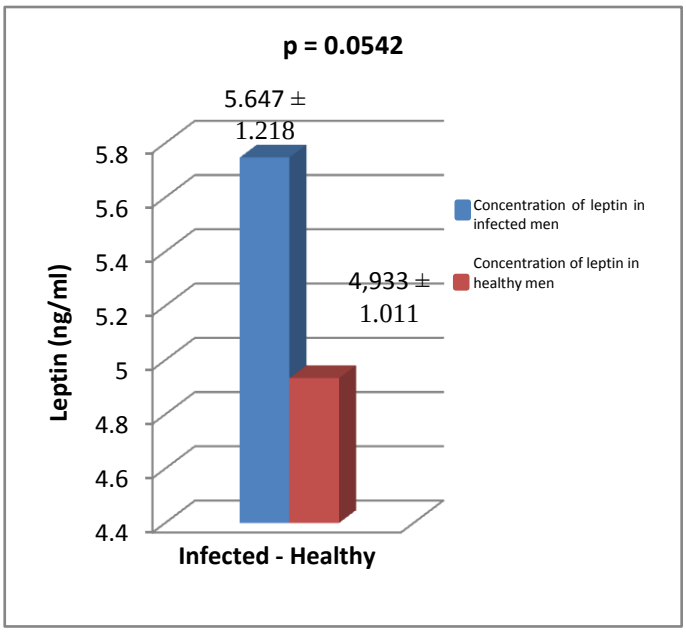

Figure 6: comparison of leptin levels in infected and healthy men.

Determnation and comparison of leptin values for infected and healthy women

The mean value ± standard deviation of leptin concentrations in affected women,was 7.769 ± 1.823 Ng/ml, and this mean value for healthy women was 6.665 ± 1.713 Ng/ml.

By applying theT-Studenttest, no statistically significant differences were observed between the mean values for these two groups (p = 0.105) (Fig 7).

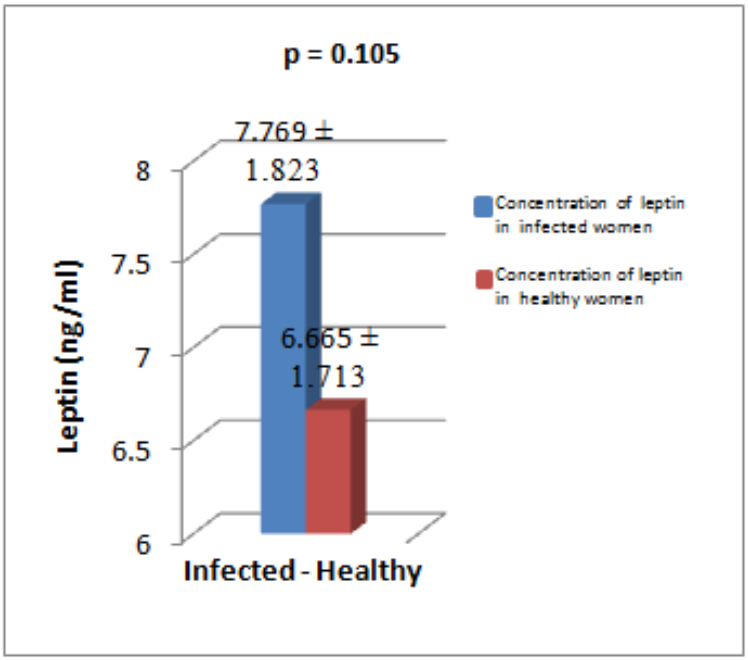

Figure 7: Comparison of leptin levels in infected and healthy women.

Determination and comparison of leptin values for infected men and infected women

The mean value ± standard deviation of leptin concentrations in infected men was5.647 ± 1.218 Pg/ml. As for the infected women, this average was 7.769 ± 1.823 Pg/ml.

By applying aT-Student test, a statistically significant difference (p = 0.000004) between the mean values of these two groups was observed (Figure8).

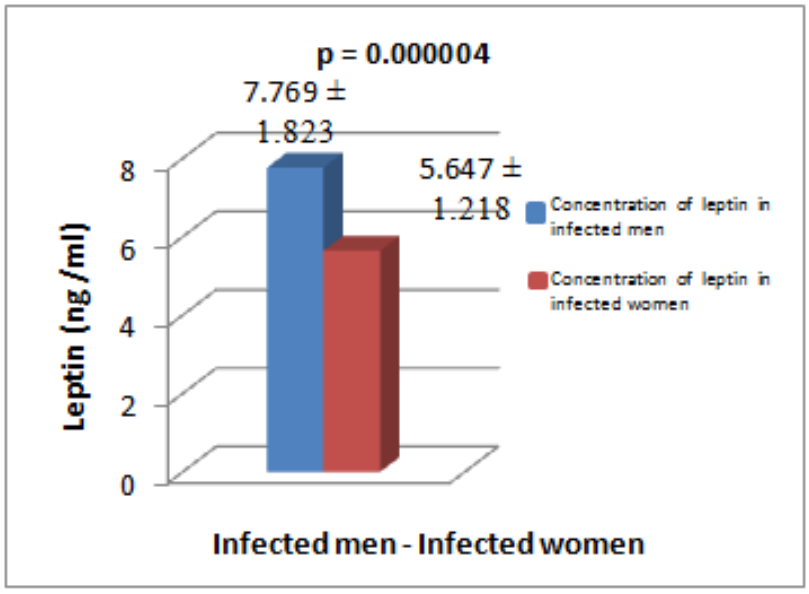

Figure 8: Comparison of leptin levels in infected men and infected women.

Study of the relationship between ghrelin and leptin in patients with H. pylori and the healthy people.
By studying the correlation relationship between ghrelin and leptin in patients with Helicobacter pylori, a statistically significant correlation was observed (correlation coefficient r= -0.314 and p= 0.01123) (Figure 9).

By studying the correlation between ghrelin and leptin in healthy subjects, no statistically significant correlation was observed (correlation coefficient r= -0.263 and p= 0.236) (Figure 10).

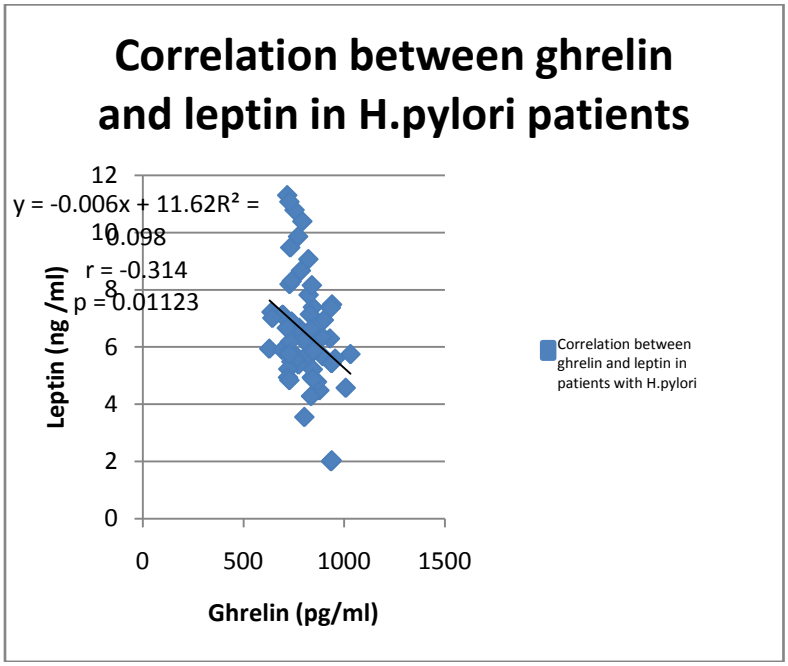

Figure 9: The correlation of ghrelin and leptin in patients with H.pylori

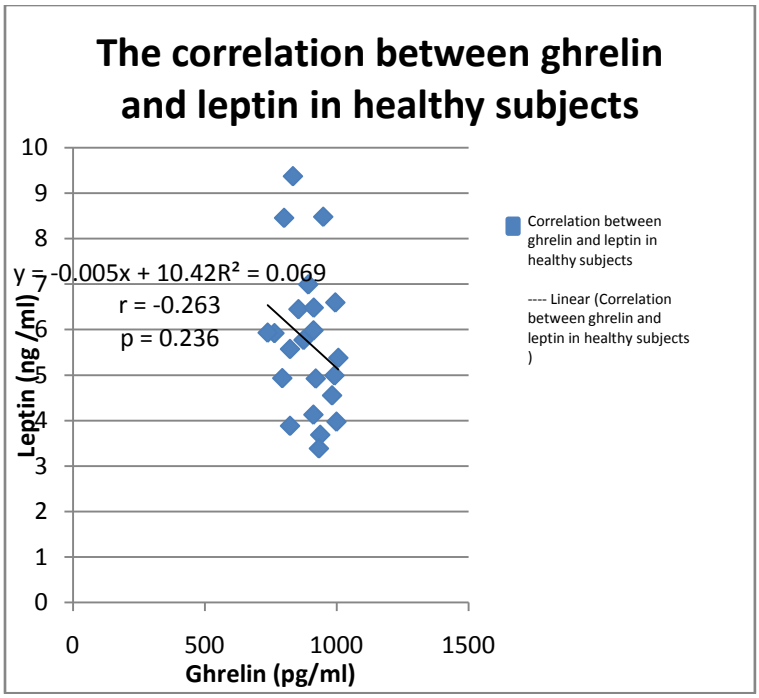

Figure 10: The correlation of ghrelin and leptin in healthy subjects.

Study of the relationship between ghrelin and BMI in H. pylori patients and healthy subjects

By studying the correlation between ghrelin and BMI in H.pylori patients, no statistically significant correlation was observed (correlation coefficient r = 0.07 and p = 0.55) (Figure 11).

By studying the correlation between ghrelin and BMI in healthy subjects, no statistically significant correlation was observed (correlation coefficient r = -0.252 and p = 0.273) (Figure 12).

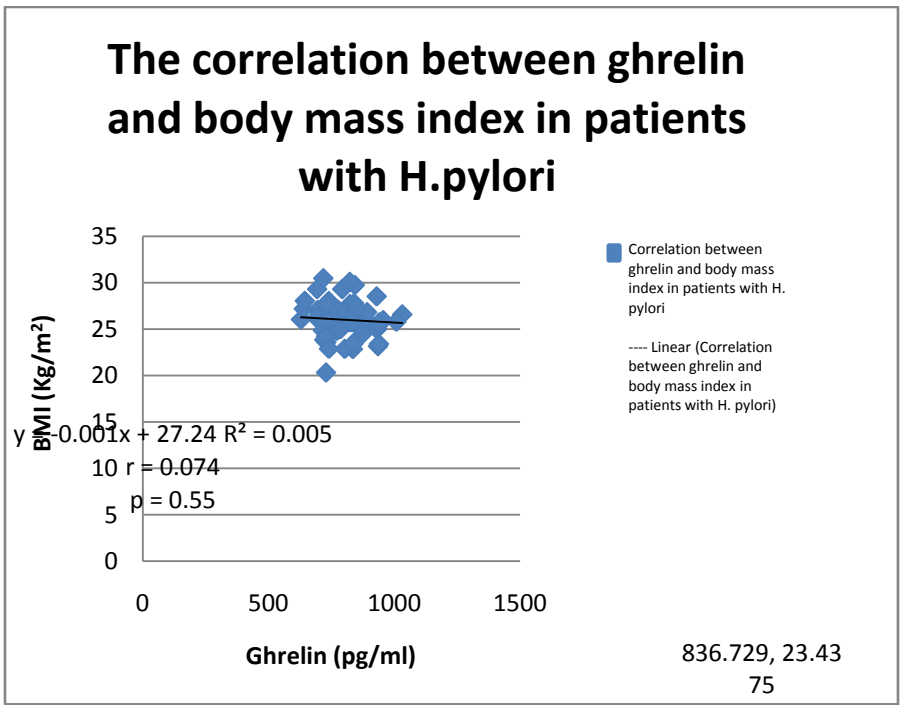

Figure 11: The correlation between ghrelin and BMI in patients of H.pylori

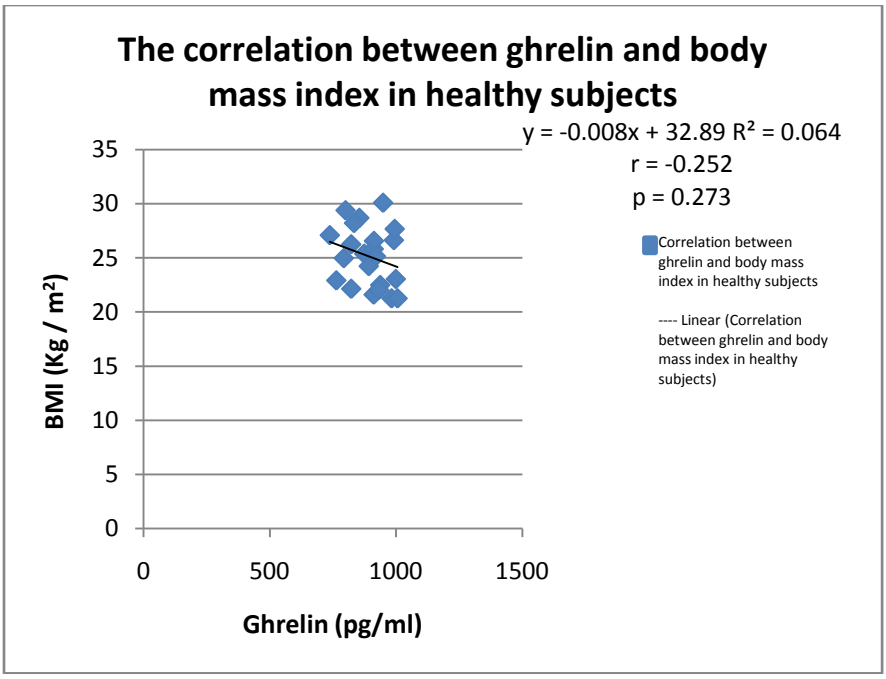

Figure 12: The correlation between ghrelin and BMI in healthy subjects

Study of the relationship between leptin and BMI in H. pylori patients and healthy subjects

By studying the correlation between leptin and BMI in patients with H.pylori, no statistically significant correlation was observed (correlation coefficient r = 0.07 and p = 0.55) (Figure 13).

By studying the correlation between leptin and the BMI in healthy subjects, no statistically significant correlation was observed (correlation coefficient r = -0.252 and p = 0.273) (Figure 14)

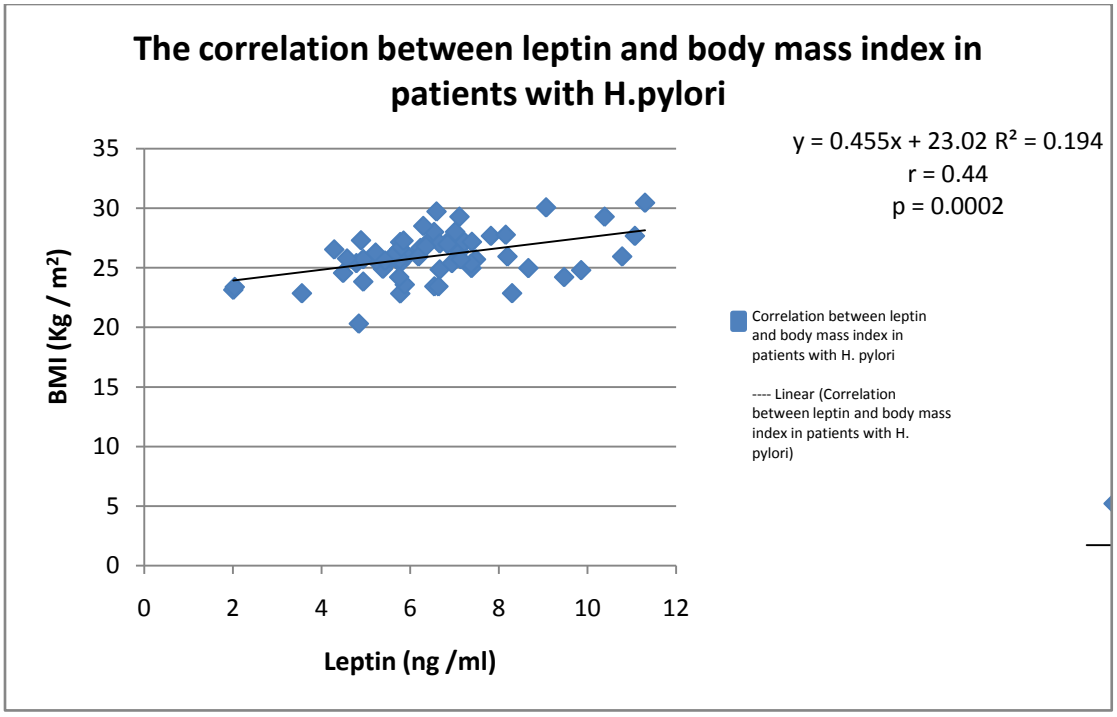

Figure 13: The correlation between leptin and the BMI in patients of H.pylori

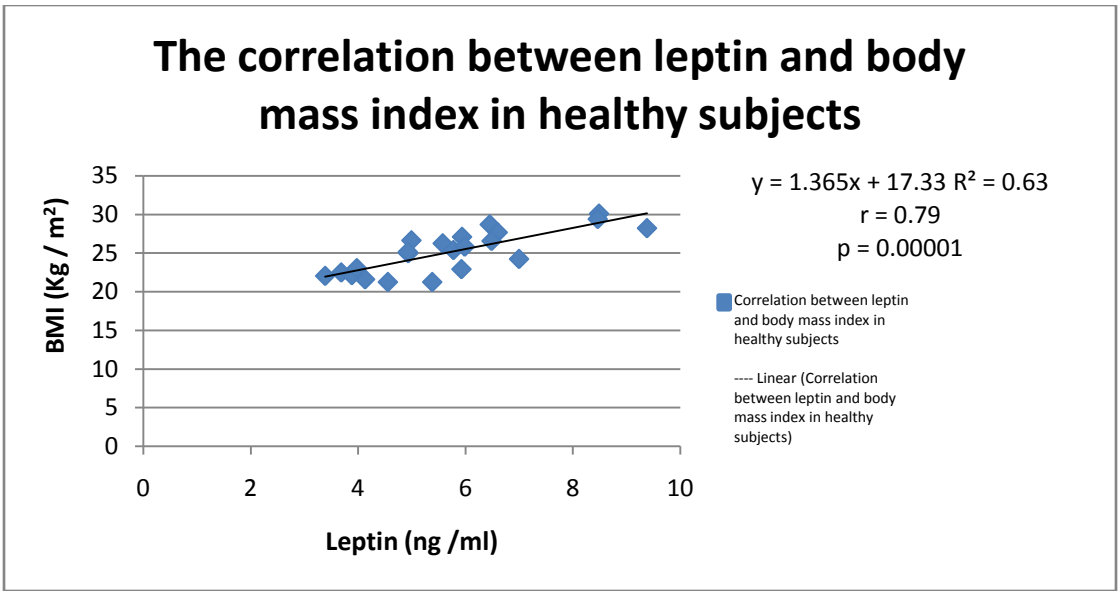

Figure 14: The correlation between leptin and the BMI in healthy subjects

DISCUSSION

1 - Ghrelin and Helicobacter pylori:

Our study showed a decrease in plasma ghrelin levels in patients compared to healthy controls. Our study also showed a decrease in plasma ghrelin levels in male patients compared to healthy males. The plasma ghrelin levels of female patients were lower than those of healthy women by a value that was not statistically significant.

This may be explained by the effect of Helicobacter pylori on the function of gastric cells producing ghrelin, as studies have shown that the excretion of gastric ghrelin messenger RNA (mRNA) is much lower in infected patients compared to healthy people, without excluding gender as an influencing factor and using the RT-PCR technique.Thus, the plasma concentrations were low as a result of decreased ghrelin production from the gastric mucosa. Research has shown that the number of ghrelin-producing cells in patients with Helicobacter pylori is significantly lower compared to healthy people, and the decrease in the number of these immune cells is accompanied by the simultaneous decrease in ghrelin plasma concentrations. This supports the statement that the plasma concentrations decrease is due to the effect of Helicobacter pylori on the function of gastric cells producing ghrelin.

The fact that H. pylori infection did not affect ghrelin levels in infected women does not mean that the previous explanation is not applicable in women but can be explained by the effect of female estrogen and the fluctuation of its levels during the menstrual cycle as estrogen can affect ghrelin production even in the case of H. pylori infection, which excludes a clear link between ghrelin and H. pylori in women.

Ghrelin concentrations were similar between the two genders in healthy people, whereas, the concentrations of ghrelin in female patients were higher than those of the male patients, and this can be explained by the effect of female hormones on the production of ghrelin and the possibility of compensating the decrease in gastric production due to bacterial infection by production from other tissues.

The results of our study agreed with:

- The study of Chiao-Hsiung Chuang et al 2009 (7), which included men and women separately, and showed a clear decrease in the level of ghrelin in infected men and did not show a change in the levels of ghrelin in the infected women. The result was explained by the germ's modification of the function of gastric cells that produce ghrelin, and by the possible effect of changes in the menstrual cycle in women in the absence of the effect of the germ on the gastric cells.
- The study of Isomoto et al. Hajime 2004 (8), which included men and women, and explained the decrease in plasma ghrelin concentrations by the effect of the gastric synthesis of ghrelin by inflammatory bacterial reactions.
- The study of Adnan Gokcel et al. 2003 (9), which included women patients, where it did not find a difference in plasma ghrelin concentrations between women patients and healthy women, and explained that the plasma ghrelin

concentrations do not directly reflect the release of ghrelin from the gastric mucosal acid-producing cells, but also represent the ghrelin produced from the other tissues.

The results of our study disagreed with:

- The study of Jatin Roper et al. 2008 (10) which included male patients and showed that Helicobacter infection did not affect plasma or gastric ghrelin, and explained this by the acclimatization of the germ-carrying host body and thus the concentrations return to their normal values, with the possibility of induction of acute production in the event of the eradication of the germ.
- The study of Yon Hoch et al. 2007 (11), which included men and women, which showed that H. pylori had no effect on plasma and gastric ghrelin levels. This was explained by the multiplicity of hormonal and nutritional factors affecting the production of gastric ghrelin and consequently plasma ghrelin concentration. The plasma leptin concentrations in women were higher than those in men, both sick and healthy. This can be explained by the fact that women have a higher percentage of lipid mass in healthy people, in addition to that in patients the fact that adipose tissue is the main source of leptin and the stomach is a secondary source of production, and thus the bacterial infection does not affect leptin concentrations between male and female patients.

2- Leptin and Helicobacter pylori:

Our study did not show a statistically significant difference in plasma leptin concentrations between H. pylori patients and healthy subjects in men and women. Research has shown an increase in the excretion of gastric leptin by the leptin-producing gastric cells in response to H. pylori infection, as it may give an "anti-inflammatory" effect, leading to the belief that this increase will be associated with an increase in plasma concentrations, but our study showed that these concentrations did not change, which can be explained by the fact that the quantities produced from the stomach are less than those produced by the adipose tissue, which represents the main source of leptin production, and therefore the effect of the germ on the production of gastric leptin will not be evident in the plasma concentrations. It can also be explained that the increase in gastric leptin only has a local effect.

The results of our study agreed with:

- The study of Chaio et al. 2009 (7), which included men and women separately, and did not show a "statistically significant" difference in the levels of leptin in male and female patients, and explained that the germ is able to alter the level of gastric leptin only, not in plasma, because only a small percentage of plasma leptin is due to gastric production.

- The study of T Azuma et al. 2009 (12), which included only infected men and women, and showed an increase in gastric leptin in response to inflammation and a decrease in it after treatment, while the serum leptin concentrations did not change before and after treatment, and explained that by the fact that the effect of leptin is local rather than systemic

- The study of Akiko Shiotaniet al. 2005 (13), which included men and women separately, and which did not show a statistically significant difference in leptin levels

The results of our study disagreed with:

- The study of Jatin Roper et al. 2008 (10), which included male patients, and showed a decrease in plasma leptin levels in patients compared to healthy people, and explained that the bacterial colonization of Helicobacter pylori inhibits gastric leptin production by causing changes in leptin-producing tissues, which modifies the inflammatory cytokine functions and leads to a decrease in circulating leptin.

3- Ghrelin and the BMI:

Our study showed that there was no inverse correlation between plasma ghrelin concentrations and body mass index (BMI) values in patients with H. pylori, and there was no inverse correlation between plasma ghrelin concentrations and BMI values in healthy subjects. This can be explained by the decrease in the production of gastric ghrelin, which was reflected in plasma ghrelin concentrations because the quantities produced from other tissues are insufficient to compensate for this decrease in gastric production.

The results of our study agreed with:

- The study of Akiko Shiotani et al. 2005 (13), which showed that there is no correlation between ghrelin and body mass index in patients.

- The study of Hajime Isomoto et al. 2004 (8), which explained the lack of correlcation with the effect of inflammatory Helicobacter pylori on the plasma ghrelin concentrations and thus on the correlation between ghrelin and BMI

4- Leptin and BMI:
Our study showed a positive correlation between plasma leptin concentrations and body mass index values in patients with H. pylori, and there was a positive correlation between plasma ghrelin concentrations and BMI values in healthy subjects. This could be explained by the inability of Helicobacter pylori to change plasma leptin levels, since the quantities produced by the stomach are small, and thus the positive relationship between leptin and BMI remains in patients as it is in healthy subjects.

The results of our study agreed with:
- The study of Jatin Roper et al. 2008 (10) and the study of Akiko Shiotani et al. 2005 (13) and the study of Hajime Isomoto et al. 2004 (8). The studies explained that adipose tissue is the main source of leptin production, and thus the relationship is not affected by the infection caused by Helicobacter pylori.

5- Ghrelin and leptin:
Our study showed an inverse correlation between plasma ghrelin concentrations and leptin concentrations in patients with Helicobacter pylori, and there was no inverse correlation between plasma ghrelin concentrations and leptin concentrations in healthy subjects.

The results of our study agreed with:
- Thestudyof Jatin Roper et al. 2008 (10), which showed an inverse correlation between plasma ghrelin concentrations and leptin concentrations in patients with H.pylori, while there was no inverse correlation between plasma ghrelin concentrations and leptin concentrations in healthy subjects.

The results of our study disagreed with:
- The study of Hajime Isomoto et al. 2004 (8), which showed that there was no inverse correlation between plasma ghrelin concentrations and leptin concentrations in patients with Helicobacter pylori, while there was an inverse correlation between plasma ghrelin concentrations and leptin concentrations in healthy subjects.

CONCLUSION

A significant decrease in plasma ghrelin levels was observed in patients with H.pylori which indicates to the importance of H. pylori as an important factor in determining ghrelin levels and the possible effect of this on BMI and the possibility of benefiting from changes in concentrations in the treatment. However, this decrease was observed in male patients only, indicating the importance of gender as a determining factor for ghrelin concentrations even in the presence of H.pylori, but no change in leptin levels was observed among patients and healthy subjects of both genders.

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