Assessment of Serum Concentration of Ghrelin and Obestatin in *Giardia lamblia* Infected Patients: A Case Control-Study

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Abstract

Background: Globally, *Giardia lamblia* is one of the major causes of diarrhea in humans and it is the commonest reported gastrointestinal parasite. Thus, the present research work aims to investigate the serum concentration of ghrelin and obestatin in patients with giardiasis. **Objective:** The current study was designed to determine of *G. lamblia* infection effects on some biomarkers such as ghrelin and obestatin. **Methods**: The study was conducted on 48 patients infected with *G. lamblia* and 48 healthy persons, who attended the general hospital of AL-Hakeem in Najaf city from September to December 2018. **The results:** The results showed a highly significant decrease (P<0.0001) in serum concentration of ghrelin and a highly significant increase (P<0.0001) in serum concentration of obestatin in *G. lamblia* infected patients (28.85ng/ml ±3.2) and (186.53ng/ml ± 1.56) respectively, in comparison to the control group (81.39ng/ml ± 3.8) and (92.89ng/ml ±2.9) respectively, with a significant positive association between the concentrations of ghrelin and obestatin (r = 0.0954). **Conclusion:** Ghrelin and obestatin are important physiological biomarkers that can support the diagnosis of *Giardia lamblia* parasite.

Keywords: *Giardia lamblia, giardiasis, ghrelin, obestatin, Diarrhea, Patients.*

Introducion

Giardiasis is a gastrointestinal infection caused by a zoonotic flagellated protozoan parasite *Giardia lamblia*. This parasite replicates in a luminal non-invasive extracellular way into the small intestine of humans and other vertebrates, that may cause a diarrheal disease [1]. Worldwide, *G. lamblia* is the third major infectious cause of diarrheal disease with more than 300 million detected cases annually [2].

Giardiasis in humans may be asymptomatic or associated with diarrhea, flatulence, abdominal cramp, malabsorption, bloating, and weight loss. Severe infection leads to malabsorptive diarrhea with bulky and greasy stools [3].

Because the gastrointestinal tract parasitic infection produces injurious and harmful effects on the tissues and the physiology of the host, various mechanisms that lead partially or completely to the loss of appetite, the loss weight, the malabsorptive diarrhea or afebrile status should be assessed [4].

Although many studies regarding intestinal protozoa as *G. lamblia* concentrate on the anorexia; to date, an appetite peptide level, ghrelin, and its derivative obestatin in the parasitic infections of the GIT have not been investigated completely.

Ghrelin is a 28-amino acid peptide, and is produced and released mainly by the stomach. Ghrelin is a main appetite regulator through initiation of meal after sensing of nutrient [5]. In addition, ghrelin control homeostasis of glucose by inhibition the secretion of insulin and control the hepatic output of glucose. Ghrelin signaling controls homeostasis of energy by reducing thermogenesis to decrease expenditure of energy. [6].

Obestatin is a 23-amino acid peptide hormone released from the stomach. Apposite to ghrelin, that causes an increase in appetite and weight (obesity), obestatin appears to act as an appetite decreasing hormone (anorectic hormone), reducing intake of food and decreasing weight gain. Obestatin also participated in ameliorating memory, controlling sleep, affecting the proliferation of cell, elevating the pancreatic juice enzyme secretion and preventing glucose-induced

insulin secretion.

Regarding the orexigenic function (appetite stimulant) of ghrelin and the anorectic effect (appetite reducer) of obestatin [7], this study hypothesized there was a reduction in the level of serum ghrelin and an increase in the serum level of obestatin in patients with giardiasis. Ghrelin is termed the 'hunger hormone' because it stimulates appetite, while Obestatin is an anorectic peptide.

In addition, the above functions of ghrelin and obestatin may affected by the change in the serum levels of these two hormones which may explain many aspects in the pathophysiology of some of the giardiasis clinical features as loss of appetite, loss of weight, absence of fever, failure to thrive in children and other signs and symptoms. Therefore, the aim of the present study was to evaluate the serum concentration of ghrelin and obestatin.

The results of the current study may supply an important information regarding the effects of ghrelin and obestatin in giardiasis.

Materials and Methods

During the period from September to December 2018, a case-control study was designed for 48 patients (28 males and 20 females) with giardiasis and 48 healthy persons, who had no clinical evidence of any type of diseases (26 males and 22 females) as a control group.

The patients were randomly selected from patients who attended AL-Hakeem hospital, Najaf Province.

Patients have included if the general stool examination was positive for *Giardia lamblia* parasite, from both genders male or female of any age.

Any patient with giardiasis on treatment, the patient with chronic loss of appetite and/or weight for any cause rather than giardiasis, febrile patient, a diabetic patient, obese patient, and/or patient with mixed intestinal parasitic infection were excluded.

The Kufa Medical College Ethical Committee approved the protocol of this study.

Stool Samples Collection: Each stool sample was collected in a clean screw cap stool containers, labeled with the number and date of collection.

Detection of *Giardia lamblia* Parasite: Microscopic diagnosis of *Giardia lamblia* was performed immediately after the collection of the stool sample. A small amount of stool specimen was processed and for each stool specimen, normal saline and Lugol's iodine direct wet preparations were performed at the same time, one slide by using normal saline (0.85%) for demonstrating the trophozoites motility and a slide with Lugol's iodine 5% for identification of internal protozoal structures [8].

Blood Specimens Collection: Five ml of blood was collected from each patient infected with *G. lamblia* and healthy person. Blood samples were drawn in sterile plain tubes and were left for 30 minutes at room temperature. Then the tubes were centrifuged for 5 minutes at 3000 rpm. Serum was collected and kept in sterile tubes in deep freeze (-20) until use.

Detection of Serological Markers: Two human biomarkers (hormones) were measured in this study. The serum concentration of the two markers; ghrelin and obestatin (Elabscience Company, Bulgaria) was determined by using ELISA technique according to the manufacturer procedure.

Statistical analysis: Data was collected, summarized, analyzed and presented using and Microsoft Office Excel 2010 and statistical package for social sciences (SPSS) version 23. The comparisons between the difference in mean concentrations of ghrelin or obestatin of the giardiasis patients and the healthy control persons were analyzed by T-test. And Spearman correlation was used to evaluate the correlation between the concentrations of ghrelin and obestatin and the results were expressed as the correlation coefficient (r). P-value less than 0.05 was considered statistically significant.

Results

The current study enrolled a total of 48 patients (28 males and 20 females) with *Giardia lamblia* infection and 48healthy persons (26 males and 22 females). Overall, the age range was from 2 years up to 71 years and 2 years up to 70 years for patients and healthy persons (control group) respectively.

The obestatin serum level of patients with giardiasis and healthy persons

The serum concentration of obestatin, in terms of mean ± standard deviation, were 186.53 ±1.56 ng/ml and 92.88 ±2.9 ng/ml in all patients with giardiasis and healthy persons respectively; with a highly significant difference (p = < 0.0001), 186.71 ±1.6 ng/ml and 93.307 ±3.1ng/ml in male patients with giardiasis and male healthy persons respectively; with a high significant difference (p = < 0.0001) and 186.28 ±1.5 ng/ml and 92.157 ±2.6 ng/ml in female patients with giardiasis and female healthy persons respectively; with a high significant difference (p = < 0.0001) as shown in figure 1.

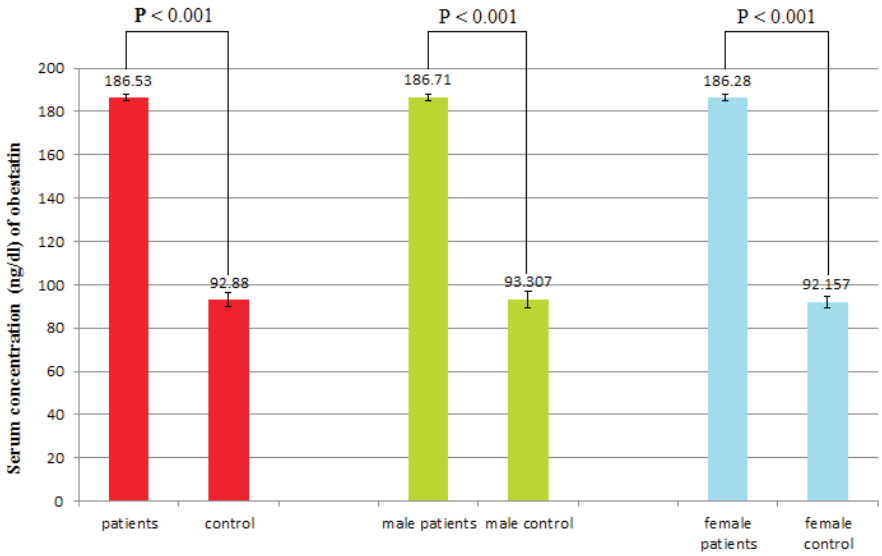

Fig. (1). Serum concentration of obestatin (mg /ml) of patients with giardiasis and control group.

The ghrelin serum level of patients with giardiasis and healthy persons

The serum concentration of ghrelin, in terms of mean ± standard deviation, were 28.848 ±3.2 ng/ml and 81.387 ±3.8 ng/ml in all patients with giardiasis and healthy persons respectively; with a highly significant difference (p = < 0.0001), 28.885 ±3.2 ng/ml and 82.521 ±3.7ng/ml in male patients with giardiasis and male healthy persons respectively; with a highly significant difference (p = < 0.0001); and 28.796 ±3.2 ng/ml and 79.800 ±3.9 ng/ml in female patients with giardiasis and female healthy persons respectively; with a high significant difference (p = < 0.0001) as shown in figure 2.

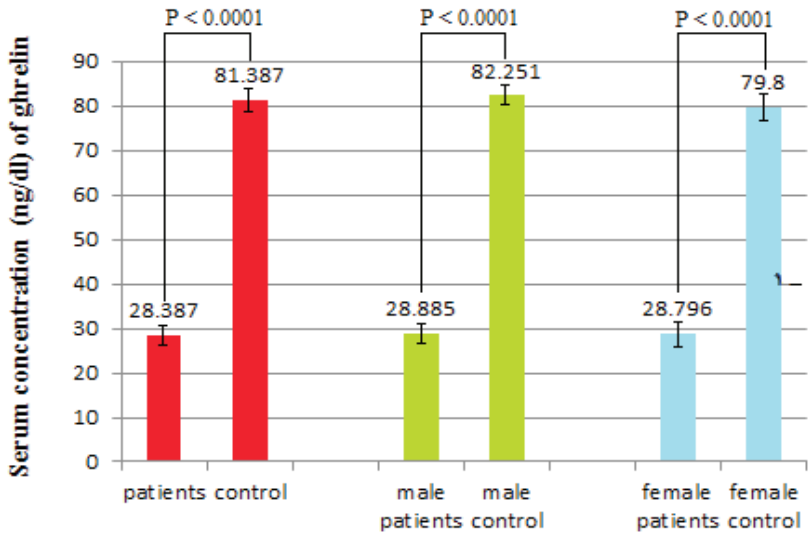

Fig. (2). Serum concentration of ghrelin (mg /ml) of patients with giardiasis and control group.

3.3. The relationship between Serum concentration of Ghrelin and Obestatin

The present study revealed a significant positive association between the serum levels of ghrelin and obestatin in *G. lamblia* infected persons (r = 0.0954) (Figure 3).

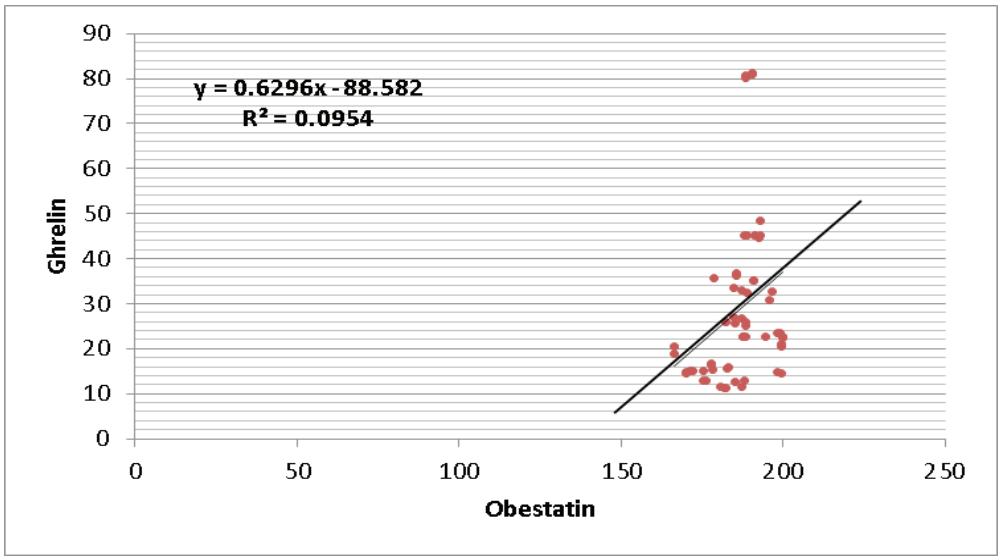

Fig. (3). The association between ghrelin and obestatin serum levels in *Giardia lamblia* infected persons.

Discussion

Giardia lamblia is an intestinal protozoan and distributes throughout the world. In 2013, the infectivity rate of giardiasis in Iraq was 1.77% and the highest rate was reported in Najaf province which was 7.9% [9]. So, *G. lamblia* infection is considered as a significant health problem in Najaf province.

The current study revealed a significantly lower serum level of ghrelin in patients with giardiasis in comparison with the healthy persons. The results of this study agree with the study of Al-Hadraawy *et al*. [5] Which proved the decrease of serum ghrelin level patients with giardiasis. In addition, the current study agrees with most studies which proved the decrease of ghrelin concentration with parasitic disease, as in the study of Ernsoy *et al*. [4].

Ghrelin level reduced in patients with *G. lamblia* infection and other protozoal and helminthic infections are suspected to recompense for a raise in the serum sugar level in these infections; explained by the association between ghrelin and insulin [10]. The reduced ghrelin level in these patients recorded in the current study is consistent with the suggestion that this may be the important cause of the anorexia in patients with giardiasis [11], also the loss of appetite could be the main cause that leads to loss of weight in giardiasis patients.

This decrease of the ghrelin may be due to the intestinal hypermotility in patients with giardiasis [11] because the protozoan *G. lamblia* colonizes in the duodenum and jeujenum by attaching to the intestinal epithelium but without any mucosal invasion [12]. The mechanism of pathogenesis is summarized by the destruction of the small intestine mucosal barrier, resulting in inflammation with malabsorption of fats [13].

Another probable reason which can interpret, at least partially, the low serum ghrelin concentrations in persons with giardiasis might be to reduce the peroxidation of lipid that elevated as a consequence of this protozoal infection [6].

The results of the current study revealed a significantly higher serum levels of obestatin in patients with giardisis in comparsion to the healthy persons, this increase could be due to parasitic damage to mucosa of intestinal in the acute period of infection such as inflammatory, ulcerative, and pathological changes in the epithelial cells villi [14]. Indeed, none of the reviewed articles study the correlation between giardiasis and serum concentration of obestatin.

This high level of obestatin is suspected to be one of the important causes beside the low level of ghrelin (as above) for the appetite loss in *G. lamblia* infected persons. The increased level of obestatin and decrease level of ghrelin reported in the current study supports what is reported by Lacquaniti *et al.,* [7] Who stated that: opposite to ghrelin, obestatin reveals as an appetite stimulant (anorectic) hormone, reducing intake of food, decreasing the emptying of the stomach and motility of jejunum, and decreasing gain of weight, and the study of Pan *et al.,* [15] who reported that the effect of obestatin on food intake is in contrast to ghrelin.

This difference in ghrelin and obestatin levels may be due to the gastric infection that associated with the severity of chronic inflammation and glandular atrophy in the corpus of the stomach [16].

The present study disagrees with the study of Tschöp *et al*., Which showed that the circulating levels of ghrelin are increased in anorexia, but obestatin is low as well as the giardia trophozoites causes an increase in the host disruption and intestinal motility [17].

In the current study, in spite of the rise in the obestatin serum concentration and the reduce in the ghrelin serum concentration in persons infected with giardiasis, the serum level of ghrelin correlated positively and significantly with the serum obestatin level. This may be explained by the fact that obestatin is a 23-amino acid peptide hormone that is derived from post-translational cleavage of preproghrelin, the same peptide precursor as ghrelin, which is a 28-amino acid peptide released from the stomach [7]. Ghrelin, a polypeptide hormone which increases appetite, and the results of this research revealed decreased levels in giardiasis. Obestatin, as an anorexigenic hormone is an important factor to fit into the complexity of the pathophysiological scenario of the protein energy wasting[18] .

Beside loss of appetite and nausea, loss of weight and failure to thrive in children may be caused by the inhibition action of obestatin on ghrelin stimulation of growth hormone levels [19], where the ghrelin is able to promote adipogenesis in the adipose tissue by directly stimulating the growth hormone secretion in the pituitary and on energy-regulating centers in the hypothalamus [20] and by making a communication between the brain and the gastrintestinal tract [21].

CONCLUSIONS

This study concluded that ghrelin and obestatin are important physiological biomarkers can support the diagnosis of *Giardia lamblia* parasitic infection. In addition, the elevation in the serum level of obestatin, and the decrease in the level of ghrelin may be the important cause of the anorexia, loss of weight and failure to grow in patients with giardiasis.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All procedures were performed in compliance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). The Kufa Medical College Ethical Committee approved the protocol of this study. Informed consent was obtained for each patient and healthy person. The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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