Mucin2 protein (MUC2), ghrelin (GHRL) and obestatin (OB) as physiological biomarkers in patients infected with *Giardia lamblia* parasite

**Shahad Dakhel Khalaf**  
College of Pharmacy/ University of Kerbala  
Email: Shahad.d@uokerbala.edu.iq

**Haider Abbas Hadi**  
Faculty of Medical Technology, The Islamic University  
Email: haider.abbas.hadi@iunajaf.edu.iq

**Ali Yas Khudair**  
Faculty of Medical Technology, The Islamic University  
Email: Ali.yas.khudair@iunajaf.edu.iq

**Rasha Saad Toma**  
Faculty of Science, University of Kufa  
Email: rr9asha@gmail.com

**Abstract**—The study was conducted on 400 suspected patients and twenty-four healthy persons, who visited the laboratory of AL-Hakeem hospital and AL-Zahra maternity and pediatrics in AL-Najaf province from October till May 2018. This study was designed to determine the effects of *Giardia lamblia* infection on some biomarkers such as (mucin-2 protein (MUC2), ghrelin (GHRL), and obestatin (OB)). whereas the numbers and percentage of the infected patient were 48 (12%) vary with different gender;28 (7%) male and 20 (5%). The result of the study revealed that concentration of (MUC2),(GHRL) and (OB) in male and female patients infected with *G. lamblia* significantly increased (P<0.05) is compared to the control group. Also, it revealed a significant increase (P< 0.05) in concentrations in the total number of patients infected with *G. lamblia* and the control group. The current study has concluded that the infection with *G. lamblia* affects some immunological and physiological markers of humans represented by (mucin-2 protein (MUC2), ghrelin (GHRL), and obestatin (OB)) as good biomarkers which may be used in the detection of *G. lamblia* parasite.

**Keywords**—giardiasis, diarrhea, MUC2, GHRL, OB, Iraq.
Introduction

Giardiasis is a gastrointestinal disease caused by a microscopic parasite called *Giardia lamblia*, proliferating in the small intestine of humans which attaches strongly to the host intestine and causes severe gastrointestinal disease [1]. This microorganism of a worldwide parasite may lead to chronic diarrhea and malabsorption in humans. Although many of the patients with *G. lamblia* parasite are asymptomatic, the main acute symptoms which appear suddenly are; diarrhea for several days, excessive gases (swelling and puff and tasting nasty taste), abdominal pain around the navel, nausea, diuretic diarrhea, and rarely vomiting or warming[2]. The cyst of this parasite can resist the unsuitable condition and adapt to the external environment to survive, whereas trophozoite is responsible for virulence properties and clinical symptomatic in the host[3].

The mucin2 protein protects epithelial cells by binding the pathogen, such as the *Giardia* parasite by disrupting the interaction between the host cells and the pathogen, it also acts to eliminate the pathogenic organism in the mucous layer and removes it by the intestinal peristalsis. Both of these actions are suitable for *G. lamblia* parasites [4]. Ghrelin is an appetite hormone secreted from the stomach, small intestine, and other organs, circulating in the blood bound to (VHDL) and (HDL), many studies on human parasitic infections recorded that the serum concentration of Ghrelin hormone changes[5]. Several studies revealed the role of human giardiasis in nutrient malabsorption and micronutrient deficiencies such as; zinc, vitamin B12, vitamin A and iron [6,7,8]. Obestatin is a peptide hormone derived from the ghrelin preprohormone and structural from 23-amino acid and released from the stomach, gastrointestinal tract, and other organs [9]. Also, this infection may lead to malnutrition, weight loss, growth impairment, and even poor cognitive development due to persistent diarrhea but not acute diarrhea [10,11,12].

Aim

This study aimed to the estimation of physiological effects in patients infected with Giardiasis due to the determination of three biomarkers levels; mucin2 protein (MUC2), ghrelin(GHRL), and obestatin (OB).

Materials and Methods

Study design and patients

From October to May 2018, 48 samples were collected from patients and 24 healthy (vary on 14 male and 10 female) who attended the clinics in AL-Hakeem hospital and AL-Zahra maternity and pediatrics in AL-Najaf province. The samples of stool were collected into clean, wide-mouth specimen bottles, from patients. The blood samples were also drawn from the same patients by vein puncture into specimen tubes to prepare the serum for estimation of the markers of study.
Diagnosis of Giardia lamblia parasite

Freshly voided stool specimens were processed and examined microscopically for intestinal parasites using the X40 objective lens as described by [13], Paniker (1989). Before a slide was considered negative, X40 objective fields of the stool smears were examined.

Serum collection

Five ml of blood are collected from healthy and infected patients. Blood samples were drawn in sterile plain tubes and left at room temperature for 30 min. Centrifuged was done at 3000 rpm for 5 minutes (Memmert, Germany). Serum was collected and kept in sterile tubes at a freeze -20 C° until use.

Serum biomarkers detection

Three human biomarkers were used in this study: mucin2 protein (MUC2), ghrelin (GHRL), and obestatin (OB). All these biomarkers kits were provided by Elabscience Company, Bulgaria, and the level of biomarkers in serum was determined by using an ELISA device (Human reader, Germany) according to the Manufacturer’s Company.

Statistical analysis

T-test was used in this study for comparison between samples by using Graphpad prism version 10 computer software. P-value less than 0.05 considered statistically significant [14].

Results

Result of study revealed that concentration of represent mucin2 protein (MUC2) and obestatin (OB) in male and female patients infected with G. lamblia were significant increase (P< 0.05) (0.130.57 ±4.04 ng. /ml), (130.11± 3.6), (186.71 ±1.6 ng /ml), (186.28± 1.5 ng /ml), respectively in compared to the control group (93.421 ± 1.8 ng. /ml), (94.079± 3.1 ng /ml), (93.307 ± 3.1 ng /ml), (92.157± 2.6 ng /ml) respectively as seen in fig.1, fig.2 and fig.3 respectively. And significant decrease (P< 0.05) (28.885 ±3.2 ng. /ml), (28.796± 3.2) respectively in compared to the control group (82.521 ± 3.7 ng. /ml), (79.800± 3.9 ng /ml) respectively as seen in fig.3.
Figure 1. Concentration of MUC2 (ng/ml) Comparison between Patients Suffering from *Giardia lamblia* Infection and noninfected Group

Figure 2. Serum concentration of obestatin (ng/dl) in noninfected Group and Patients Suffering from *Giardia lamblia* Infection
Figure 3. Concentration of ghrelin (ng/ml) Comparison between Patients Suffering from *Giardia lamblia* Infection and noninfected Group.

**Relationship between Serum Levels of Ghrelin and Obestatin**

The present study revealed that the levels of serum concentration of (ghrelin) correlated positively and significantly with the obestatin level in the serum of patients with *G. lamblia* \( r = 0.0954 \), as seen in Figure 4.

![Figure 4. The Correlation between Serum Concentrations of ghrelin and obestatin in patients suffering from *Giardia lamblia* infection.](image)

**Discussion**

The results of the study showed a significant increase in the serum level of mucin 2 in male and female patients infected with *G. lamblia* parasite compared with the control group, also in total patients with *G. lamblia* compared to the total control group, this increase may be due to pathophysiological responses of *Giardia* trophozoites which lead to the development of diarrheal disease, disruption mucosal layer of the human intestine as well as a malabsorptive
diarrheal disease [15]. and may be due to the mechanism of Giardia infection or intestinal inflammation where the mucin 2 protein plays an important role in the protection of intestinal tissue in addition to its ability to stimulate immunity and body production of cellular motions and the assembly and calling of white blood cells to the site of injury, which stimulates the mucous intestinal glands to increase the secretion of mucus to compensate for the shortfall caused by infection Giardia parasites [16]. The mucin2 protein protects epithelial cells by binding the pathogen, such as the Giardia parasite by disrupting the interaction between the host cells and the pathogen, it also acts to eliminate the pathogenic organism in the mucous layer and removes it by the intestinal peristalsis. Both of these actions are suitable for G. lamblia parasites [4]. agrees with the study of [17].

The increase in the concentration of OB could be due to intestinal parasitic infections which may cause damage to intestinal mucosae such as inflammation, ulceration, and pathological changes in the villi of epithelial cells in the acute period of infection [18], and the obestatin hormone released from epithelial cells and mucosa lining the stomach and small intestine, Obestatin derived from the same prohormone as ghrelin, participates in the decrease of gastrointestinal motility, appetite inhibition, weight loss, inhibits gastric emptying and jejunal motility and increased memory function in mammals. These roles are dissimilar to the functions of ghrelin [19,20]. Giardia trophozoites lead to the development of diarrheal disease as the infection causes malabsorption and diarrhea due to reducing water intake and have demonstrated that in addition to a pharmacologic action to inhibit water drinking [21,15].

The results of the present study agree with the study [22], that showed an elevated obestatin level through acute pancreatitis with elevated ghrelin level in this disease he also showed no relation between ghrelin and obestatin levels in the case of acute pancreatitis. The results of the study revealed a significant decrease in the serum level of GHRL in male and female patients infected with G. lamblia parasite compared with the control group, also in total patients with G. lamblia compared with the control group, the decrease in ghrelin concentration in the patients observed in this study is compatible with the suggestion that this could be the main cause for the loss of appetite in patients with parasitic infections [23]. This decrease may be due to the hypermotility of the intestine in humans infected with the Giardia parasite [24], because its pathogen colonizes the small intestine and can attach to the epithelium but does not invade the mucosa [25]. In addition, gastrointestinal infections inhibited ghrelin production from gastric endocrine cells, leading to lower plasma and gastric mucosal ghrelin levels[26]. This is because ghrelin is released from tissues including the small and large intestines[27], and plays central and peripheral biological roles in food intake, gastric motility and acid secretion, and major factors that influenced plasma ghrelin levels [28]. The reduction of ghrelin levels may be due to the anorexia symptom caused by G .lamblia infections [29]. The results of the present study agree with the study of [30].

Conclusion

The pathogenic effect of G .lamblia in patients infected with recurrent diarrhea leads to changes in serum levels of son biomarkers such as humane mucin2
protein (MUC2), ghrelin (GHRL), and obestatin (OB) as a good biomarker may be used in the detection of *G. lamblia* parasite.

**Acknowledgments**

The authors thank Kufa University for providing the required facilities to carry out this research work and Hospital for gifting us the serum samples from their patients.

**Conflict of interest**

The authors assert that there is no conflict of interest.

**Financial support and sponsorship**

There is no financial support and sponsorship.

**References**

5. Chuang CH; Sheu BS; Yang HB; Lee SC; Kao AW; Cheng HC; Chang WL; Yao WJ. (2009). Gender difference of circulating ghrelin and leptin concentrations in chronic Helicobacter pylori infection. Helicobacter;14:54–60.


15. Manko A; Motta J-P; Cotton JA; Feener T; Oyeyemi A; Vallance BA; et al. (2017) Giardia co-infection promotes the secretion of antimicrobial peptides beta-defensin 2 and trefoil factor 3 and attenuates attaching and effacing bacteria-induced intestinal disease. University of Calgary PLoS ONE 12(6).


18. Olusi SA; Al-awadhi C; Abiaka M; Abraham ; George S. (2003). Serum copper levels and not zinc are positively associated with serum leptin concentrations in the healthy adult population. Biol Trace Elem Res; 91: 137-144.


25. Saleem Khteer Al-Hadraawy;Mohammed Emad Al-Ghurabi; Malak Maged Almusawi ; Mohammad Alzeydi (2016). Ghrelin And Melatonin As Biomarkers In Patients With Giardiasis University Of Kufa ; 553-5.


