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Evaluation of Serum Ghrelin and Leptin Levels in Patients with Celiac Disease, Type1Diabetes Mellitus and Rheumatoid Arthritis

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Abstract

Ghrelin and leptin are two hormones that possess multiple functions, including appetite regulation, maintenance of the tissue homeostasis and regulation of proinflammatory cytokines. A few studies on serum ghrelin and leptin levels in autoimmune diseases have exhibited conflicting results. Therefore, the present study aimed to investigate the relationship between the two energy balance hormones and autoimmune diseases. Serum ghrelin and leptin levels were assessed in 94 adult patients, 61 females and 33 males, with various autoimmune diseases (celiac disease, type 1 diabetes mellitus and rheumatoid arthritis) as well as in 35 healthy people as controls, using commercially available ELISA kits. Statistically important distinctions ($P < 0.05$) were found between the patients and controls with regard to serum ghrelin and leptin levels. Moreover, females had higher mean serum ghrelin and leptin levels than males. On the other hand, serum ghrelin level was positively correlated with serum leptin levels ($r = 0.399$, $P < 0.05$) in the RA group. Whereas no significant correlation ($P > 0.05$) was found between serum ghrelin and leptin levels in both CD and T1DM groups. As well as the correlation of the diseases biomarkers (tissue transglutaminase antibodies, anti-tTG; glutamic acid decarboxylase antibodies, anti-GAD; and cyclic citrullinated peptide antibodies, anti-CCP) with ghrelin/leptin levels revealed that anti-CCP was the only marker that significantly ($P < 0.05$) associated with ghrelin and leptin in patients with RA. The current study indicates a linkage between the immune system and metabolic hormones depending on response to different autoimmune conditions. Additional studies are required to understand whether changes in ghrelin-leptin levels influence the emergence of autoimmune diseases or vice versa.

Keywords: Ghrelin, Leptin, Celiac disease, Type 1 diabetes mellitus, Rheumatic arthritis

تقييم مستويات الغريلين واللبتين في مصول مرضى حساسية القمح والنوع الأول من داء السكري والتهاب المفاصل الرثوي

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الخلاصة

يُعد الغريلين واللبتين من الهرمونات ذات الوظائف المتعددة ، بما في ذلك تنظيم الشهية ، والحفاظ على التوازن الداخلي لأنسجة الجسم وتنظيم الحركيات الخلوية المحرضة على الالتهابات. لقد أظهرت بعض الدراسات التي أجريت على مستويات هرموني الغريلين واللبتين في الدم في أمراض المناعة الذاتية نتائج متضاربة. لذلك هدفت الدراسة الحالية إلى معرفة العلاقة بين هرموني توازن الطاقة (الغريلين واللبتين) وأمراض المناعة الذاتية من خلال تقييم مستويات هذين الهرمونين في مصول (94) مريضًا بالغًا ، 61 أنثى و 33 ذكرًا ، يعانون من أمراض المناعة الذاتية المختلفة (حساسية القمح ، داء السكري من النوع 1 والتهاب المفاصل الرثوي) وكذلك لدى (35) شخصًا أصحاء (مجموعة سيطرة)، باستخدام عُدد الأليزا المتاحة تجاريًا. تم العثورعلى تباينات مهمة إحصائياً ($P < 0.05$) بين مجاميع المرضى و مجموعة السيطرة فيما يتعلق بمستويات هرمون الغريلين واللبتين في مصول من شملتهم الدراسة. اضافة الى ذلك ، كان متوسط مستويات هرمون الغريلين واللبتين لدى الإناث أعلى من الذكور. ومن ناحية أخرى ، ارتبط مستوى الغريلين بشكل إيجابي مع مستوى اللبتين في مجموعة RA ($r = 0.399$، $P < 0.05$) ، في حين لم يتم العثور على ارتباط معنوي ($P > 0.05$) بين مستويات هرمون الغريلين واللبتين في كل من مجموعات حساسية القمح والنوع الأول من داء السكري . فضلاً عن ذلك، فإن دراسة الارتباط بين الدلالات المناعية للأمراض (مضادات tTG ، ومضاد GAD ، ومضاد CCP) مع مستويات الغريلين / اللبتين كشفت أن الدلالة الوحيدة التي ارتبطت بشكل كبير مع الغريلين واللبتين كانت مضاد CCP في مرضى التهاب المفاصل الرثوي. تشير الدراسة الحالية إلى وجود صلة بين الجهاز المناعي والهرمونات الأيضية اعتمادًا على الاستجابة لأمراض المناعة الذاتية المختلفة، وان هناك حاجة لدراسات إضافية لفهم ما إذا كان التغير في مستويات هرمون الغريلين و اللبتين يؤثر على ظهور أمراض المناعة الذاتية أم العكس.

Introduction

Autoimmune diseases are often systemic conditions that affect various body structures and organs. There are currently 80–100 diseases that are known to develop as a result of autoimmune responses and they impact about 10% of the world population [1].

Celiac disease (CD) is an autoimmune inflammatory illness of the small intestine which is characterized by the presence of autoantibodies triggered in response to gluten exposition in genetically susceptible individuals, resulting in intestinal inflammation and disturbances in energy metabolism [2]. Measurement of antibodies against tissue transglutaminase (tTG) is the most reliable test for CD [3]. Type 1 diabetes mellitus (T1DM) is another chronic autoimmune illness in which insulin-producing pancreatic beta cells are destroyed, causing an extreme insulin deficit and hyperglycemia [4]. Antibodies against glutamic acid decarboxylase (anti-GAD) are reliable T1DM serological indicators [5]. Furthermore, rheumatoid arthritis (RA) is a chronic autoimmune disease that mostly affects the synovial joints. Tumor necrosis factor alpha (TNF-a) is suggested to be a key cytokine in the development and propagation of inflammation by stimulating osteoclastogenesis, and thus inducing bone erosion [6]. Previous studies have shown that anti-cyclic citrullinate peptide (anti-CCP) antibody test provide a greater sensitivity and specificity in diagnosing rheumatoid arthritis [7].

Recent studies have been linked to different bioactive peptides such as ghrelin and leptin with multiple metabolic as well as immune effects. In 1999, ghrelin was recognized to be an endogenous ligand for the growth hormone secretagogue receptor (GHS-R). It is a 28-amino-acid peptide produced mostly by X/A-like enteroendocrine cells in the gastrointestinal tract and serves as a potent circulating orexigenic hormone controlling food intake, energy expenditure, obesity and releasing growth hormone (GH) [8]. The distribution of functional ghrelin receptors (GHS-R) within a wide variety of tissues including lymphoid organs suggests a link between the immune system and systemic metabolism in response to different physiological and

pathological conditions [9]. Ghrelin is a potent anti-inflammatory mediator in lymphocytes, monocytes and dendritic cells. It stimulates IL-10 expression and cell migration while inhibiting oxidative stress, cellular apoptosis, cell adhesion and proinflammatory cytokine expression [10]. Thus, changes in ghrelin levels can directly affect immune responses and tissue homeostasis [11, 12] . However, little is known regarding the immunomodulatory role of the orexigenic hormone ghrelin in immune dysfunction and autoimmunity [13].

On the other hand, leptin, another 16 kDa peptide hormone of the long chain helical cytokine family, is mainly produced by white adipocytes [14]. Since its discovery in 1994, leptin has piqued the scientific community's interest for its pleiotropic activities. It has a dual role as a hormone and a cytokine. It controls food intake and energy homeostasis as a hormone. While as a cytokine, leptin enhances inflammatory reactions [15]. The relationship of the anorexigenic hormone leptin with the immune system has been widely studied [16]. Importantly, leptin has been shown to play a significant role in T-lymphocyte responses. Without doubt, leptin represents a connection between metabolism, nutritional status and immune responses [17]. Moreover, there has been a growing evidence that leptin has a role in the pathogenesis of different autoimmune disorders [18-20]. Both high and low levels of leptin may play a role in autoimmune diseases. It has also been suggested as a potential therapeutic target in autoimmune diseases treatment [21].

Despite strong evidence of the effect of ghrelin and leptin on immune system, only limited data is available regarding the correlation between serum ghrelin and leptin levels in autoimmune disease patients. Their exact immunomodulatory role has also been controversial. Therefore, this study aimed to determine the possible relationship between the two energy balance hormones (ghrelin and leptin) and autoimmunity by evaluating serum ghrelin and leptin levels in patients with some autoimmune diseases in comparison with healthy people.

Materials and Methods

Patients

After ethical committee approval, a total of 94 adult patients (61 females and 33 males) attended medical laboratories in Basrah Province, Iraq. The subjects aged between 18–40 years, with a confirmed diagnosis of selected autoimmune diseases (28 patients with CD, 33 patients with RA, and 33 patients with T1DM), were enrolled in this study from May 2020 till August 2021. Another 35 apparently healthy people (volunteers, college students and health workers) without any evidence of autoimmune diseases matched to the cases by age, gender and body mass indices (BMI 18.3–27.7 kg/m²) were also enrolled as a control group.

Laboratory Assays

Blood samples were collected from all participants following fasting for 10 hours. Then, sera were separated and stored at -20°C until later analysis. Using standard methods, immunological laboratory tests were performed including: tTG-IgA (Auskulisa/Germany), GAD-IgG (Euroimmun/UK) and CCP-IgG (Auskulisa/Germany) to confirm the diagnosis of CD, T1DM and RA respectively.

Serum Ghrelin and leptin levels were measured by commercially available Enzyme Linked Immuno Sorbent Assay (ELISA) kits (MyBiosource/USA for Ghrelin and LDN/Germany for Leptin), following the manufacturer's guidelines.

Statistical Analysis

The data was analyzed by SPSS (Statistical Package of Social Sciences) software. Estimation of the differences between variant groups was made with independent *t* test, one-

way ANOVA and Pearson correlation. All data was expressed as mean±SD and a *P* value of < 0.05 was considered to be significant.

Results

The demographic and laboratory characteristics of patients with CD, T1DM and RA as well as controls have been presented in Table 1. The mean ± SD age of patients with CD, T1DM and RA were 33.03±6.20, 28.48±5.51and 32.15±6.39 years respectively. Whereas those of controls were 32.42±6.84 years. No statistically significant variations were found between the patients and controls, with regard to age except in T1DM group, where they differed significantly ($P < 0.05$). However, females were predominating in all groups.

Table 1: Demographic characteristics and laboratory findings in patient groups & the control group

Groups & Parameters	Patient (mean ±SD)	Control (mean ±SD)	*P* -value
CD (n=28, 10 ♂/18♀)		**(n=35, 12 ♂/ 23♀)**	
▪ **Age**	33.03±6.20	32.42±6.84	0.717
▪ **Anti-tTG IU/ml**	43.51±24.77	--------	--------
▪ **Ghrelin pg/ml**	478.96±133.89	266.32±29.88	0.000
▪ **Male**	383.50±137.90	269.70±31.563	0.022
▪ **Female**	498.38±132.50	264.56±29.54	0.000
▪ **Leptin ng/ml**	10.13±3.84	7.07±2.78	0.001
▪ **Male**	6.74±1.32	4.30±1.417	0.000
▪ **Female**	10.85±4.58	8.66±3.77	0.102
T1DM (n=33, 12 ♂/21♀)		**(n=35, 12 ♂/ 23♀)**	
▪ **Age**	28.48±5.51	32.42±6.84	0.011
▪ **Anti-GAD IU/ml**	32.13±14.74	---------	----------
▪ **Ghrelin pg/ml**	221.94±64.78	266.32±29.88	0.001
▪ **Male**	229.67±54.32	269.70±31.56	0.038
▪ **Female**	217.52±70.95	264.56±29.54	0.009
▪ **Leptin ng/ml**	5.71± 2.42	7.07± 2.78	0.036
▪ **Male**	4.22± 1.67	4.30± 1.41	0.907
▪ **Female**	6.56± 2.39	8.52± 2.14	0.007
RA (n=33, 11 ♂/22♀)		**(n=35, 12 ♂/ 23♀)**	
▪ **Age**	32.15±6.39	32.42±6.84	0.868
▪ **Anti-CCP IU/ml**	29.93±12.34	---------	---------
▪ **Ghrelin pg/ml**	531.39±192.05	266.32±29.88	0.000
▪ **Male**	383.50±137.90	269.70±31.56	0.022
▪ **Female**	605.34±173.10	264.56±29.54	0.000
▪ **Leptin ng/ml**	10.33±4.32	7.07± 2.78	0.001
▪ **Male**	6.74± 1.32	4.30± 1.41	0.000
▪ **Female**	12.12± 4.19	8.52± 2.14	0.001

Serum ghrelin levels in the patient groups of CD and RA significantly ($P < 0.05$) elevated as compared with the healthy controls (478.96±133.89 and 531.39±192.05 respectively *vs* 266.32±29.88 pg/ml). While the levels significantly ($P < 0.05$) demoted in patients with T1DM (221.94±64.78 *vs* 266.32±29.88 pg/ml) (Table 1; Figure 1).

As well as patients with CD and RA had significantly ($P < 0.05$) higher mean leptin level than the control group (10.13±3.84 and 10.33±4.32 respectively *vs* 7.07±2.78 ng/ml). However, serum leptin levels were remarkably ($P < 0.05$) lower in patients with T1DM than of control group (5.71±2.42 *vs* 7.07±2.78 ng/ml) (Table 1; Figure 2).
Ghrelin levels were also found to be comparable, statistically non-significant differences, between CD and RA patients, whereas significant ($P < 0.05$) difference was found in T1DM group in comparison with CD and RA patient groups (Table 2; Figure1). Similar statistical results were obtained for leptin values as pointed out in (Table 2; Figure 2).

Table 2: Mean levels of ghrelin and leptin in the serum of patients with CD, T1DM, RA and control group.

Groups	CD (n= 28)	T1DM (n= 33)	RA (n= 33)	Control (n=35)	*P* value
Ghrelin (Mean pg/ml ±SD)	478.96±133.89 a	221.94±64.78 b	531.39±192.05 a	266.32±29.88 c	0.000
Leptin (Mean ng/ml±SD)	10.13±3.84 a	5.71±2.42 b	10.33±4.32 a	7.07±2.78 c	0.000

Different letters refer to: the mean difference is significant at the 0.05 level.

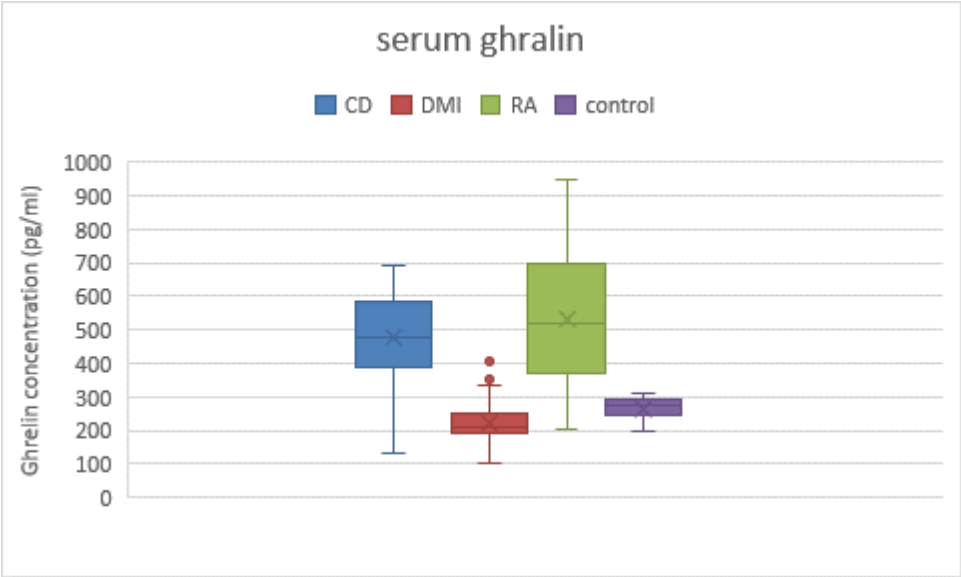


Figure 1: Serum ghrelin mean level in in patients with CD, T1DM, RA and control group.

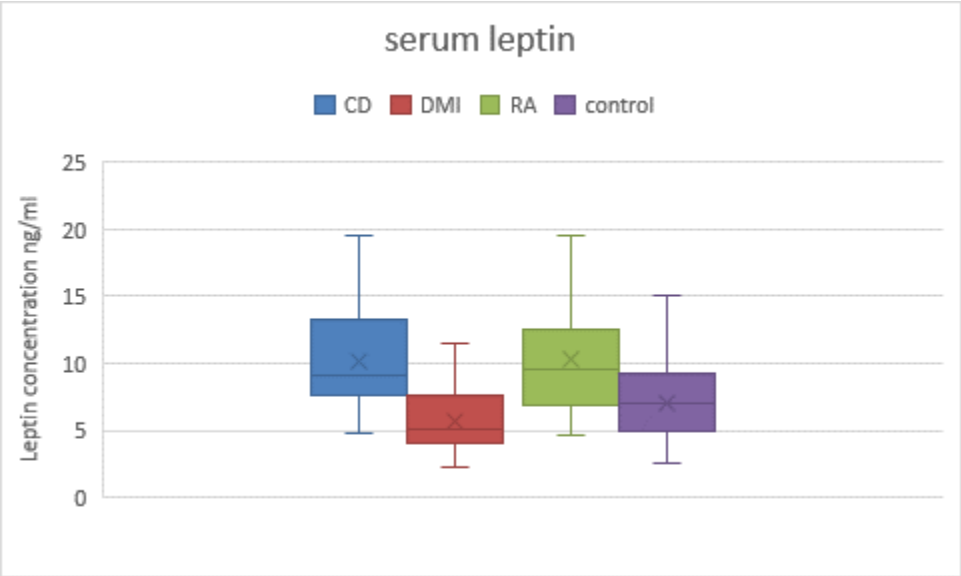


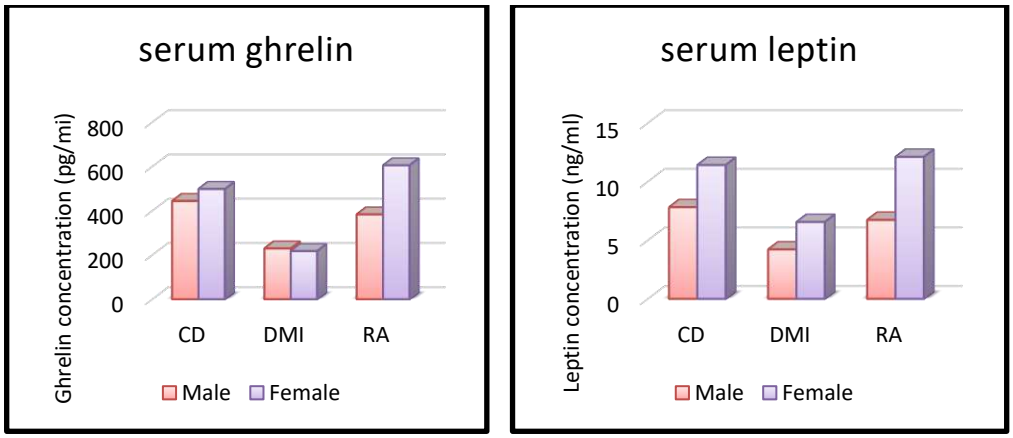
Figure 2: Serum leptin mean level in patients with CD, T1DM, RA and control group.

With respect to the gender, there were no statistically significant ($P > 0.05$) variations between male and female patients with CD and T1DM in mean serum ghrelin levels. Whilst only in RA patients, the difference between males and females remained significant ($P < 0.05$) (383.50±137.90 *vs* 605.34±173.10 ng/ml, respectively) (Table 3, Figure 3a). Furthermore, the mean serum leptin levels were significantly ($P < 0.05$) higher in females than in males with CD, T1DM and RA (Table 3, Figure 3b). Additionally, Table 3 shows a comparison of serum ghrelin and leptin mean levels among patient groups with respect to each gender. Regarding males, T1DM group revealed significant ($P < 0.05$) differences in mean serum levels of both ghrelin and leptin in comparison with CD and RA groups, while statistically non-significant ($P > 0.05$) variations were found between CD and RA groups. Likewise, this result was found in females with regard to the leptin values, however ghrelin values exhibited significant contrast among all patient groups.

Table3: Comparison of serum ghrelin and leptin mean levels between males and females within each patient group (rows) & among patient groups with respect to each gender (columns).

Groups	Ghrelin (mean pg/ml ±SD)			Leptin (mean ng/ml±SD)		
	Male	Female	*P* value	Male	Female	*P* value
CD	444.00±136.04 a	498.38±132.50 a	0.312	7.82±2.44 a	11.42±3.92 a	0.006
T1DM	229.67±54.32 b	217.52±70.95 b	0.612	4.22±1.67 b	6.56±2.39 b	0.006
RA	383.50±137.90 a	605.34±173.10 c	0.001	6.74±1.32 a	12.12±4.19 a	0.000
P value	0.000	0.000		0.000	0.000	

Different letters refer to: the mean difference is significant at the 0.05 level.



(a) (b)

Figure 3: (a) Comparison of serum ghrelin mean levels in patients with CD, T1DM & RA with respect to the gender. (b) Comparison of serum leptin mean levels in patients with CD, T1DM & RA with respect to the gender.

Correlation between serum ghrelin and leptin levels in different patient groups are illustrated in Table 4. No significant relationships were observed between serum ghrelin and leptin levels in both CD and T1DM groups (r = 0.339, P = 0.078; r = 0.026, P = 0.886 respectively). Yet there was a weak relationship between serum ghrelin and leptin levels in RA group (r = 0.399, P < 0.05).

Table 4: Correlation between serum ghrelin and leptin levels in different patient groups.

Patient Groups	Ghrelin-Leptin	
	***P* value**	***r* value**
CD	0.078	0.339
T1DM	0.886	0.026
RA	0.021	0.399

In addition, Table 5 shows the correlation of the diseases serological markers (anti-tTG, anti-GAD and anti-CCP) with serum ghrelin and leptin levels. It was found in the study that anti-CCP antibody was the only serological marker with significant (P <0.05) correlation with both ghrelin and leptin levels positively (r= 0.625; r= 0.574, respectively). Whereas no correlations were observed with anti-tTG and anti-GAD.

Table 5: Correlation of anti-tTG, anti-GAD and anti-CCP with serum ghrelin and leptin levels

Serological Markers	Ghrelin		Leptin	
	***P* value**	***r* value**	***P* value**	***r* value**
Anti-tTG	0.161	0.272	0.758	0.061
Anti-GAD	0.054	0.338	0.531	0.113
Anti-CCP	0.000	0.625	0.000	0.574

Discussion

Current study was conducted to investigate the relationship between the two energy balance hormones (ghrelin and leptin) and autoimmune diseases. Statistically significant differences in mean ghrelin and leptin serum levels were found between different patient groups and controls. Regarding CD patients, serum ghrelin levels were higher in patients with CD compared with controls. Similar results were obtained by other studies [22-24] which evaluated ghrelin levels

in adults with CD. In addition to Selimoglu *et al*., who reported increasing ghrelin levels in childhood CD [25]. Additionally, increasing evidence points to ghrelin immunoregulatory function which suppresses inflammation and promotes an anti-inflammatory profile [9]. Otherwise, serum leptin levels were also higher in patients with CD when compared to controls in this study. Less data is available on leptin in patients with CD, in which the results disagree with the results of current study. Boguszewski *et al*., Ertekin *et al*. and Qassim *et al*. concluded that serum leptin levels markedly reduced in patients with CD compared to the controls [26-28]. While Russo *et al*. have shown that serum leptin concentrations were the same in adult CD patients and in healthy adults [29]. On the other hand, this study showed that both ghrelin and leptin levels were significantly lower in type 1 diabetic patients than those of the control group. There are limited reports related to ghrelin and leptin levels in patients with type 1 diabetes. However, our results support other published studies [30-33] that found low ghrelin and/or leptin levels in patients diagnosed with T1DM and infer that could be a protective mechanism against hyperglycemia. Furthermore, in RA patients, mean serum ghrelin and leptin levels were significantly higher when compared with the healthy controls. In line with the findings of this study, significantly elevated serum levels of ghrelin or leptin in patients with RA have been reported in some studies [34-36], while others found decreased levels [37-40]. Reasons for these discrepancies in results may be due to the differences in sample size, variability of the baseline characteristics of entrants (age, gender, race, disease duration, BMI, …), utilization of different methods to evaluate ghrelin and leptin levels, or underlying patients therapies that interfere with the endocrine system

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Moreover, statistical analysis, in this study, showed significant rise in serum ghrelin and leptin levels in female patients when comparison was made according to gender. However, for patients with CD and T1DM, the differences in ghrelin did not reach statistical significance between male and female patients. In general, the majority of autoimmune diseases are more common in females than in males [41]. Females are thought to mount stronger humoral and cellular immune responses than males, which may influence their vulnerability to autoimmune diseases [42]. Ortona *et al*. found that sex hormones play a critical role in gender bias, with estrogens acting as potent stimulators of autoimmunity and androgens acting as protective hormones [43]. However, the newest theories for what causes sex differences in autoimmune diseases pointed to a complex set of interactions between hormones, X chromosomes and other physiological factors [44].

Otherwise, the correlation between mean serum ghrelin and leptin levels in different patient groups were also studied. Although in CD and RA both of serum ghrelin and serum leptin were increased and both of them were decreased in T1DM. No significant relationships were observed between serum ghrelin and leptin levels in both CD and T1DM groups. Whereas there was a weak relationship between them in RA group. According to previous studies, ghrelin and leptin are thought to have mutually reciprocal regulatory actions within the immune system. Ghrelin has been suggested to exert multiple immunomodulating effects [10]. It has also been demonstrated to reduce leptin-induced pro-inflammatory reactions in human mononuclear and T cells [11]. Whilst leptin has been demonstrated to improve immune reactions in autoimmune diseases that are usually associated with inflammatory responses and the effects of leptin vary considerably from one pathophysiological condition to another [17, 45]. Thus, the variations in circulating levels of leptin and ghrelin may have a considerable impact on the production of various cytokines by immune cell populations. In this study, the unidirectional ghrelin and leptin effects may disrupt immune cell homeostasis, resulting in aberrant cytokine production, which may play a role in the autoimmune diseases.

In addition, the correlations of diseases biomarkers (anti-tTG, anti-GAD and anti-CCP) with ghrelin and leptin serum levels were also investigated. Anti-CCP antibody was the sole biomarker with significant correlation, whereas no correlations were observed with anti-tTG and anti-GAD. Previous literatures on RA reported a correlation between leptin and some clinical and inflammatory markers, including anti-CCP antibodies. Gómez-Bauelos *et al*. found that leptin production enhanced in pre-obese and obese RA patients with anti-CCP status, suggesting that leptin may be an important mediator for maintaining autoimmune humoral responses in RA, particularly in anti-CCP positive patients [46]. In the context of an autoimmune conditions such as RA, Gupta *et al.* found that leptin stimulates B cells and plasma cells to create higher anti-CCP antibodies titers [47]. In 2019, Porchas-Quijada and colleagues also reported a positive correlation between anti-CCP antibodies and total anti-ghrelin autoantibodies when they investigated the correlation between the clinical parameters and metabolic profile in RA, suggesting an increase in the affinity of these autoantibodies toward ghrelin [48].

Conclusion

The current study indicates the presence of an obvious difference between patients and healthy groups with regard to serum ghrelin and leptin levels, and suggests a linkage between the immune system and metabolic hormones depending on response to different autoimmune conditions. Additional studies are required to understand whether changing in ghrelin-leptin levels influence the emerge of autoimmune diseases or vice versa.

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