Effects of Iron Deficiency Anemia and its Treatment on Ghrelins, Obestatin and Heat Shock Protein 70

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Abstract: The impact of iron deficiency anemia (IDA) and its treatment on increased levels of heat shock protein 70 (HSP70) in settings with higher tissue stress induced by both ghrelin, which is both an antioxidant and a food intake stimulant, and also obestatin with opposing effects were investigated. The association of pica with these parameters was also examined. The study included 28 patients with IDA and 28 healthy controls. While acyl ve des-acyl ghrelin values were lower ($p<0.05$) in IDA. With treatment, ghrelin levels climbed. In IDA, obestatin levels were higher than the control values ($p<0.05$). With the IDA treatment, acyl and des-acyl Ghrelin levels increased. Contrarily, obestatin values fell down. The concentration of HSP 70 in IDA and during its therapy was above control values. Acyl, des-acyl ghrelin, obestatin, and HSP70 levels were increased in the pica group. In the pica group obestatin/acyl ghrelin ratio was comparatively higher ($p<0.05$). In IDA decrease in ghrelin and an increase in obestatin levels are observed, while HSP 70 remains the same. An increase in the obestatin/acyl ghrelin ratio might be responsible for the pica disorder.

Keywords: Iron deficiency anemia, ghrelin, obestatin, heat shock protein 70.

INTRODUCTION

The human body has a positive correlation between iron stores and ghrelin levels. In the prodromal phase of iron deficiency anemia (IDA), a decrease in appetite is expected—subjective scores related to appetite and food intake increase following treatment of IDA. Iron therapy induces the synthesis of ghrelin and nesfatin-1 in the human body, thus causing increased appetite and food intake [1-4]. Eating disorders, obesity, and cachexia threaten millions of people around the world. Physiologically, the organism's short-term food intake control is regulated by the gastrointestinal tract, hypothalamus, and obesity hormones such as leptin, adiponectin, resistin, ghrelin, and nesfatin-1 released from many parts of the body. It is thought that ghrelin and nesfatin-1 are key in regulating appetite, food intake, energy expenditure, and body weight in the short and long term. Lower levels of Ghrelin in IDA might cause pica disorder due to its impact on eating habits. Ghrelin is a peptide produced in the gastrointestinal tract. Ghrelin stimulates the pituitary release of the growth hormone and is involved in the hypothalamic regulation of energy homeostasis. Ghrelin demonstrates a pivotal role in energy homeostasis. Low ghrelin levels increase calorie intake. Fasting conditions such as cachexia and anorexia nervosa are determined to be high ghrelin levels. As an anti-inflammatory agent and immune-regulating hormone/cytokine, ghrelin may be valuable in treating inflammatory diseases. There are two circulating forms of ghrelin; acyl ghrelin and des-acyl ghrelin. Acyl ghrelin is considered the metabolically active fraction of the hormone that regulates appetite. Ghrelin O-acyl transferase was recently discovered as the enzyme responsible for the hormone acylation and activation. Desacyl ghrelin has long been considered the degradation product of ghrelin without biological activity. However, recent evidence suggests it behaves like a separate hormone and might be a functional inhibitor of ghrelin. Furthermore, according to some studies, higher acyl/des-acyl ghrelin ratios are linked to obesity and hyperphagia [4,5].

Obestatin opposes the effects of ghrelin and suppresses weight gain [6]. In the literature, any study related to obestatin level in IDA has not been encountered.

Heat shock proteins (HSPs) are induced by various stressors in order to confer protection against such stressors [7]. Normally, levels of HSP are decreased. Rise in the ambient temperature, ischemia, hypoxia, enhanced (blood) pressure, heavy metals, free oxygen radicals, inflammation, hormones, antibiotics, cytokines, and in cases of intensive stress like infections might increase levels of HSP. Under stressful conditions, mostly heat shock protein 70 (HSP70) is affected [8,9]. The release of HSP 70 is stimulated by

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E-ISSN: 1929-4247/23

ghrelin. The exact levels of HSP70 in IDA are unknown, while its concentration rises in hypoxia and at higher ambient temperatures [8-10]. In the literature, any study concerning levels of HSP70 in IDA has not been encountered [7-9,11,12].

The impact of IDA and its oral treatment on increased levels of HSP70 in settings with higher tissue stress induced by both ghrelin, an antioxidant and a food intake stimulant, and obestatin, with opposing effects, were investigated. The association of pica with these parameters was also examined.

MATERIALS AND METHODS

Between March 2018 and March 2019, 28 cases with IDA were monitored in the Department of Pediatric Hematology outpatient clinics, and 28 healthy control subjects referred to the polyclinics of the Department of Healthy Children whose blood samples had to be obtained to exclude anemia were enrolled in the study. Informed written consents were obtained from children comprising IDA and control groups and their families.

Cases diagnosed as IDA were categorized into pre- (Group a) and post-treatment (Group b) groups (shown in Table **1**). Hb concentrations below the values adjusted for age groups (4 months–2 years, <10.5 g/dl, 2–6 years, <11.5 g/dl, 6–12 years, <12 g/dl, and 12–18 years, <12 g/dl) were considered as indicators of Hb deficiency. IDA was diagnosed in cases with decreased Hb, SI, TS (<16%), and F (<12 ng/ml) values [13].

The samples were taken in a fasting state between 08:00-09:00 AM. Blood samples were taken from cases diagnosed as IDA before initiation of treatment and at 3rd month of the therapy to perform complete blood counts (CBC), differential counts, reticulocyte counts,

Table 1: Demographic Data and Hematologic Values of the Patients and Control Groups

	Iron Deficiency Anemia n= 28		Control (c) n= 28	p<0.05
	Before Treatment (a) n= 28	After Treatment (b) n= 28		
Age median (min-max)	6.45 (1-16)	6.75 (1.3-16.3)	8.55 (1-16)	—
Weight median (min-max)	19.4 (8.4-56)	20.3 (9.3-56.6)	24.9 (9-65.7)	—
Height median (min-max)	113.7 (66.5-172)	116 (73-173.5)	130.2 (77-160)	—
Gender n (%)	14 M (50) 14 F (50)	14 M (50) 14 F (50)	15 M (53.5) 13 F (46.5)	
Hb g/dl, median (min-max)	9.6 (5.2-1.5)	12.3 (11-14.6)	13.6 (12.1-14.8)	a-b, a-c
Fe µg/dl, median (min-max)	10 (3.0-29.6)	41 (19-225)	87 (36-185)	a-b, a-c
TIBC µg/dl, median (min-max)	358 (251-463)	287 (129-430)	261.5 (187-382)	a-b, a-c
F ng/ml, median (min-max)	4.0 (1.5-11.5)	26.6 (13.4-139)	30.9 (14.2-174)	a-b, a-c
TS %, median (min-max)	2.94 (1.22-8.6)	13.6 (5.7-174)	29.7 (11.1-82.8)	a-b, a-c

Hb: Hemoglobin, **MCV**: Mean corpuscular volume, **RDW:** Red cell distribution width, **Fe:** Serum iron, **F:** Ferritin, **TIBC:** Total iron binding capacity, **TS:** Transferrin saturation.

measurements of serum iron, and total iron uptake capacity (TIBC). Also, systemic physical exams were conducted together with the determination of erythrocyte sedimentation rate (ESR) and levels of C-reactive protein (CRP) so as to exclude any possibility of infection. In every milliliter of the blood sample, 20-30 µl protease inhibitor aprotinin was added. On samples of plasma obtained following centrifugation, 1 N HCL was added at an amount of one-tenth of the volume of the sample used. The samples were stored at - 20 with - 80 ºC and processed simultaneously. Plasma ghrelin, obestatin, and HSP 70 were analyzed with Peptide Enzyme Immunoassay (EIA) method and evaluated in compliance with the kit manufacturer's catalog. For the analysis of ghrelin, for des-acyl Ghrelin (Cat#A05119), human acyl ghrelin (Cat#A05106) ELISA Kit (Bertin Pharma, Cayman Chemicals, USA) was used. Obestatin was analyzed in a Biotec L800 analyzer using Human Obestatin ELISA kit (BACHEM AG, Germany Cat#S-1284). HSP 70 was studied by Biotec L800 analyzer using HSP 70 High Sensitivity EIA Kit (Assay designs Stressgen, Brussels, Belgium; Cat.#EKS-715). Currently, all available manufacturers' ghrelin assays appear analytically acceptable, although switching among the manufacturers within one study is not recommended. Therefore, a comparison of peptide concentrations should be analyzed with the same company kits. Intra-assay and inter-assay coefficient of variation (CV) for the des-acyl ghrelin was 4.40% and 4.50% at room temperature, respectively, while Intra-assay and inter-assay CV for the acyl ghrelin were 6.20% and 6.70% at room temperature, respectively. The inter-assay and intra-assay CV for obestatin was 9.6% and 8.6%, respectively, while the inter-assay and intra-assay CV for HSP70 were 3.9% and 3.2%, respectively.

Cases diagnosed as IDA received a Fe^{+2} formulation at a daily dose of 4 mg/kg given at 2-3 equal dosages on an empty stomach.

Table 2: Demographic Data and Hematologic Values in the Pica and Non-Pica Groups

	Iron Deficiency Anemia Before Treatment		**p<0.05**
	Pica n= 9 (32.1%)	**Non-Pica n= 19 (76.9%)**	
Age year, median (min-max)	2.6 (1-14)	10 (1-16)	+
Height cm, median (min-max)	86.7 (66.5-154.5)	134.5 (71-172)	+
Weight kg, median (min-max)	12.5 (8.4-41)	30 (8.7-56)	+
Temperature °C, median (min-max)	37 (36.5-37.2)	37 (36.3-37.3)	–
Hb g/dl, median (min-max)	9.8 (7.3-11.5)	9.5 (5.2-11.4)	–
Fe µg/dl, median (min-max)	7 (3-15)	12 (5-29.6)	–
TIBC µg/dl, median (min-max)	297 (251-409)	376 (261-463)	–
F ng/ml, median (min-max)	3.4 (1.5-10.8)	4.5 (1.5-11.5)	–
TS ng/ml, median (min-max)	2.4 (1.2-4.1)	3.3 (1.3-8.6)	–

– = $p<0.05$.

For statistical analysis, SPSS for Windows 12.0 software program was used. For the assessment of study data, descriptive statistical methods (median, min-max) were used, and the Mann-Whitney-U test was employed to compare quantitative data. In contrast, *chi*-square and Fisher's exact test were utilized to compare qualitative data. Pre-and post-treatment comparisons of the cases were conducted using Wilcoxon Signed Rank test, and $p<0.05$ was considered to be statistically significant.

Our university's Scientific Research Project Unit (University of Firat Faculty of Medicine) provided financial support (Approval: FÜBAP-1669) for our study.

RESULTS

Tables **1** and **2** show the demographic data of the cases and the hematologic values obtained. IDA treatment increased acyl and des-acyl ghrelin levels while obestatin concentrations fell. Though not statistically significant, values of HSP70 were found to be at their peak levels following IDA therapy, while in the control group, they declined to their nadir (Table **3**, Figure **1**).

IDA detected a difference between mean body temperatures measured during pre- and post-treatment periods ($p<0.05$). The mean body temperature (a-before treatment 37.0, 36.3-37.3, b-after treatment

Table 3: Levels of Acyl and Des-Acyl Ghrelin, Obestatin, and HSP 70 in the IDA and Control Groups; the Pica and Non-Pica Groups

	Iron Deficiency Anemia			p<0.05
	Before Treatment (a)	After Treatment (b)	Control (c)	
Acyl ghrelin pg/ml, median (min-max)	57.5 (35.5-101)	82 (34.5-132)	71.5 (33-119)	a-b, a-c
Desacyl Ghrelin pg/ml, median (min-max)	555.5 (339-988)	800.5 (368-295)	683.5 (346-135)	a-b, a-c
Obestatin ng/ml, median (min-max)	7.3 (4.3-23.2)	5.6 (3.7-18)	5.5 (3.7-13.1)	a-b, a-c
HSP 70 pg/ml, median (min-max)	0.39 (0.2-1.11)	0.38 (0.2-1.33)	0.34 (0.21-1.02)	-
	Before Treatment (a) Pica n= 9	**Before Treatment (a) Non-Pica n= 19**		
Acyl ghrelin pg/mL, median (min-max)	59 (44.5-96)	54 (35.5-101)		-
Desacyl Ghrelin pg/ml, median (min-max)	657 (440-988)	491 (339-949)		-
Obestatin ng/ml, median (min-max)	7.8 (4.7-15.2)	6.8 (4.3-23.2)		-
HSP 70 pg/mL, median (min-max)	0.52 (0.25-0.87)	0.29 (0.2-1.11)		-
Obestatin/Acyl ghrelin pg, median (min-max)	132 (105.6-158.3)	125.9 (121-229.7)		+

+ = $p<0.05$, — = $p<0.05$.

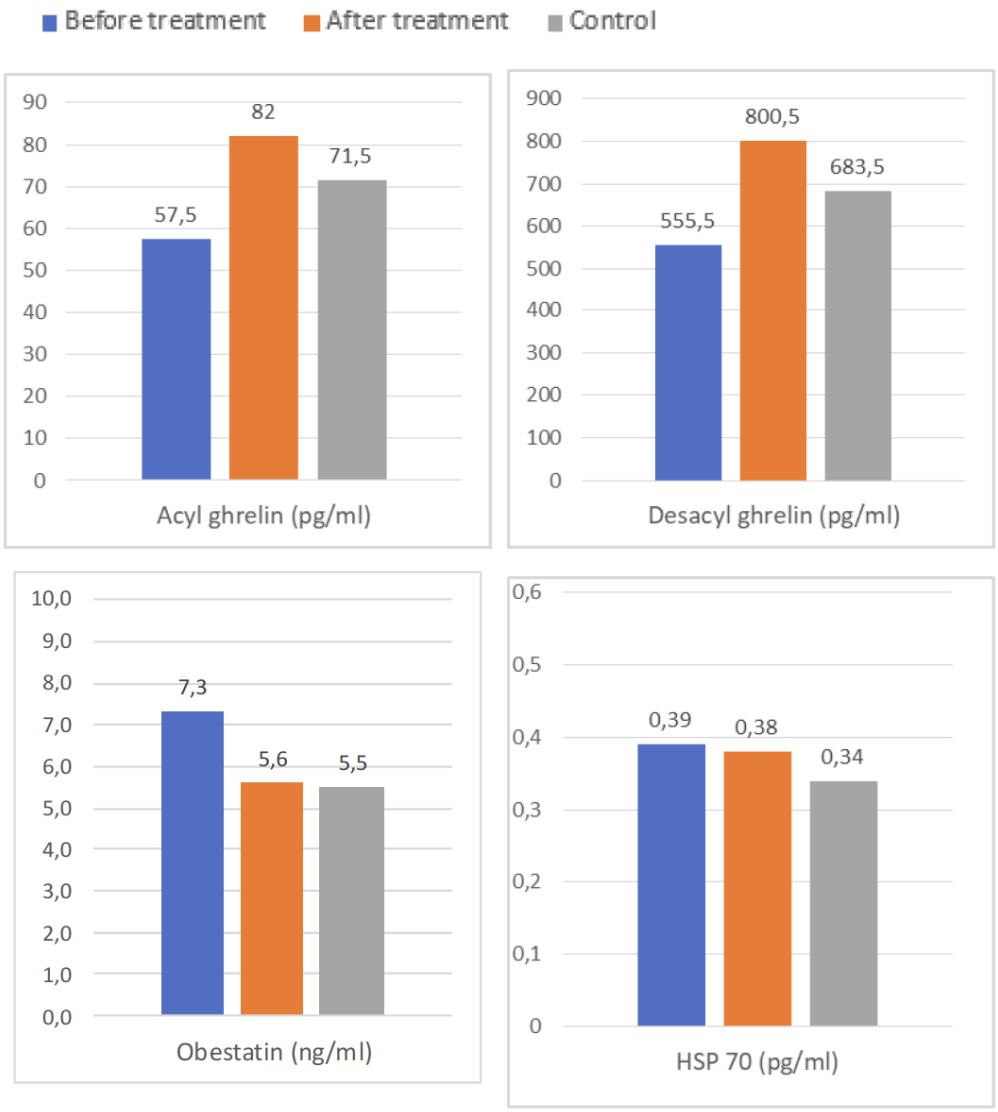

Figure 1: Levels of acyl and des-acyl Ghrelin, obestatin, and HSP 70 in the patient and control groups.

37.0, 36.8-37.4) and the number of pica (a-before treatment n= 9, b-after treatment n= 0) cases were statistically different between the patient and control (c-37.0, 36.7-37.4 groups ($p<0.05$, a-b, a-c). In the Pica group, the patients' mean age, height, and body weights were comparatively lower ($p<0.05$). However, mean body temperatures measured in the Pica group did not differ from those obtained in the healthy control group. Hematologic values in the Pica group did not differ from those obtained in the non-Pica group (Table **2**). Despite a lack of significance, relatively higher acyl, des-acyl ghrelin, and HSP70, but lower obestatin concentrations were detected in the Pica group. Obestatin/acyl ghrelin ratio was 132, while in the Non-Pica group, it was detected to be 125.9 ($p<0.05$). The ratios of obestatin/des-acyl ghrelin were 11.8 and 8 in the Pica, and the control groups, respectively ($p<0.05$). In the Pica group, the ratio of obestatin to acyl, des-acyl ghrelin, and HSP 70 was relatively higher (Table **3**, Figure **2**).

DISCUSSION

IDA is a deficiency state affecting infants' and children's quality of life and performance levels [12]. Loss of appetite is one of its clinical features [14]. Appetite is under the control of cerebral functions [15,16]. Ghrelin is known as an appetite-regulating hormone [6,17]. Peripheral ghrelin administration stimulates food intake [18] and secretion of growth hormone [6] and leads to increases in body mass indices [6,19-23]. Levels of ghrelin increase following weight loss and decrease after weight gain [24].

IDA is associated with decreased appetite. The ghrelin hormone is one of the major regulators of appetite. IDA patients have a reduced appetite and

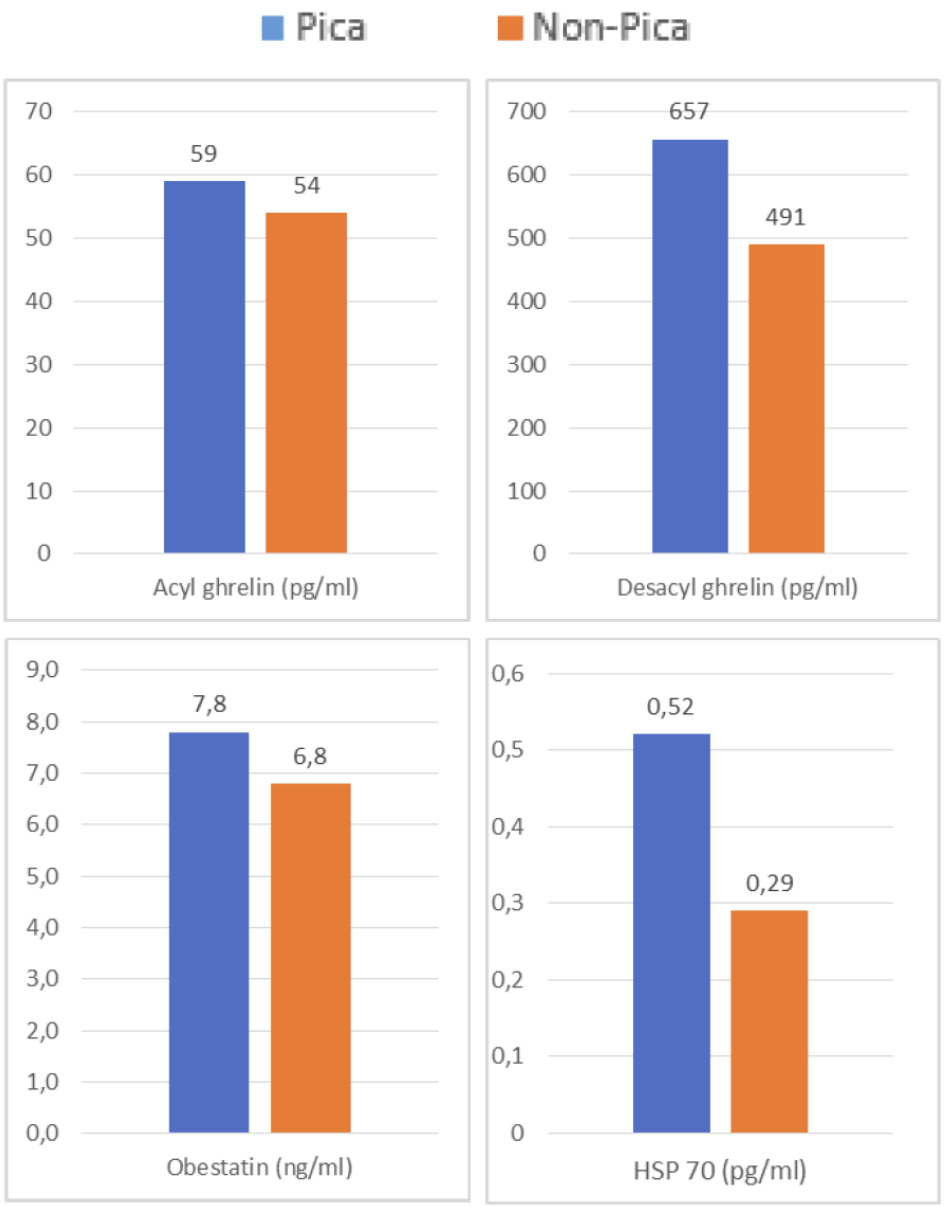

Figure 2: Levels of acyl and des-acyl Ghrelin, obestatin, and HSP 70 in the Pica and non-Pica groups.

ghrelin hormone activity. Treating IDA enhances appetite and ghrelin levels (Table **3**). A subjective decrease in food intake and appetite is observed in children diagnosed with IDA [4]. Pica was made according to the anamnesis. In our study, since we couldn't objectively assess the food intake, the degree of increase in appetite during the post-treatment period could not be evaluated.

The level of ghrelin decreases progressively from the state of tolerable deficiency in iron stores toward IDA [1]. In our study, levels of acyl and des-acyl ghrelin in cases diagnosed as IDA were significantly lower in the before-treatment and control groups ($p<0.05$). With a 3-month treatment, an insignificant amount of weight gain was observed. Our findings suggest that ghrelin might contribute to the development of IDA. In IDA, lower levels of ghrelin which is recognized as a stimulator of food intake, remind us of its potential role among etiologic factors in the development of IDA. Do decreased levels of ghrelin lead to pica disorder and alterations in eating habits? The answers to these questions are not clear yet.

Desacyl ghrelin is found in its free form in circulation, while an important part of acyl ghrelin is bound to large molecules as lipoproteins [25]. Ghrelin plays a crucial role in regulating lipid metabolism [26]. It inhibits lipolysis and stimulates the proliferation and differentiation of preadipocytes [27]. Assuming potential

future changes in the amount of adipose tissue, and blood lipid profile owing to weight loss, relatively lower levels of acyl ghrelin in IDA were foreseen. In our study, acyl and des-acyl ghrelin fractions in the IDA group were lower when compared with the post-treatment period and the control group. Besides, a significant increase in post-treatment ghrelin level was observed ($p<0.05$) (Table **3**, Figure **1**).

The range of measurement for ghrelin differs among manufacturers of kits. Measurements performed using various manufacturers' kits differ as much as 10-fold. Normal ranges of reference values for acyl and des-acyl ghrelin are 32.61-65.2 pg/ml, and 300-430 pg/ml, respectively [28]. Values obtained from our cases, including the control group, are quite above these reference ranges (Table **3**, Figure **1**). In this study, one type of ghrelin manufacturer's kit was used, so the assays used here were analytically acceptable.

During the development of IDA, plasma ghrelin levels decrease parallel with iron stores decrements. Ghrelin might influence not only nutritional status but also eating habits [1]. Reduced levels of Ghrelin in IDA might be associated with the development of pica. We wanted to know if the impact of pica is responsible for further decreases in the concentrations of acyl and des-acyl ghrelin forms. The relevant effects of obestatin/acyl ghrelin and obestatin/des-acyl ghrelin ratios have been investigated. In our study, 9 cases of pica disorder were detected among patients diagnosed with IDA. With a 3-month treatment, all cases were rendered pica-free. Any difference concerning hematologic parameters of IDA patients with or without pica was not detected (Table **2**). The lack of cases might preclude the detection of any significant difference between both groups. In addition, any difference between groups of IDA with or without pica was not observed for acyl and des-acyl ghrelin values (Table **3**). Non-observance of any difference might be due to psychologic, cultural, and pharmacologic (iron, copper, selenium, zinc, calcium, magnesium, blood lead) factors involved in the etiology of pica [29]. Alteration in obestatin/acyl ghrelin and obestatin/des-acyl ghrelin ratios might play a role.

In rats, peripheral ghrelin administration protects against gastric and reperfusion damage and decreases serum LDH and TNF-α levels. Ghrelin has antioxidant activity [30]. Alteration in the antioxidant state might be associated with a decrease in ghrelin levels in cases diagnosed as IDA [31]. Oxidative stress increases in IDA.

Central or peripheral ghrelin administration results in dose-related increases in body temperature. Although the underlying cause of this body temperature alteration is unknown, it might be explained by the role of ghrelin in energy consumption and conservation [32]. Following treatment of IDA, body temperature rises significantly ($p<0.05$). An increase in the concentrations of both forms of ghrelin might explain this rise in body temperature. Though there was a lack of any significance in the post-treatment group, the mean ghrelin concentration was comparatively higher than the control group ($p>0.05$) (Table **3**).

Obestatin and ghrelin are coded by the same gene and suppress weight gain. However, obestatin has an anti-ghrelin activity [33,34]. It slows down gastric emptying. Inhibition of jejunal contractions triggers afferent vagal signals resulting in a centrally induced satiety response [6]. In our study, obestatin levels remarkably peaked during the pretreatment period of the cases diagnosed as IDA, and they were relatively higher than those measured both in the post-treatment period and also in the control group (trough levels) ($p<0.05$) (Table **3**). Higher levels of obestatin in IDA suppress food intake and, together with lower levels of ghrelin, contribute to the development of nutritional IDA. We reviewed the literature and could not find any study evaluating both ghrelin and obestatin in IDA. The ratio of obestatin to ghrelin might be more important.

HSPs are a family of proteins induced in cells exposed to different insults. This induction of HSPs allows cells to survive stressful conditions. Mammalian HSPs have been classified into six families according to their molecular size: HSP100, HSP90, HSP70, HSP60, HSP40, and small HSPs, including HSP27. These proteins act as molecular chaperones, either helping in the refolding of misfolded proteins or assisting in their elimination if they become irreversibly damaged. In the case of endogenous hyperthermia, alterations in HSP70 value were detected. After heat shock or other metabolic stress, HSPs are expressed at high levels in all tissues and cells. Various HSPs have pro-survival functions, including chaperone, anti-apoptotic, and/or anti-inflammatory activity. HSPs holds a dual role depending on their location. Inside cells, they fulfill essential survival functions as molecular chaperones forming complexes with intracellular polypeptides (self or foreign) to help in protein folding, the resolution of protein aggregates, and intracellular protein transport. Released from the cell, they act as messengers communicating the cells' interior protein composition to the immune system for initiation of

immune responses against intracellular proteins. Clinical hyperthermia might be a way for HSP70 to induce its immunologic activity via local necrosis. Heat can also cause necrosis, allowing the release of HSPs to the extracellular environment. Among the different HSPs, HSP70 is the one that is most strongly induced by heat. HSPs would not only announce the presence of danger but also inform the immune system about the enemy's identity [35]. In febrile states, HSPs are induced [8,9]. IDA increases the predisposition to hypothermia [36]. During tissue stress, levels of ghrelin ve HSP70 are altered [13,37,38]. Hypoxemic symptoms predominate if anemia develops because of rapid blood loss [31,39]. Levels of HSP70, which increase hyperthermia, have been observedly unchanged in IDA, predisposing to hypothermia. In cases diagnosed as IDA, the patients' body temperatures increase significantly ($p<0.05$). Even if peak values of HSP 70 were to be obtained during the post-treatment period, any significant difference between both groups could not be detected. Also, in the pretreatment group, mildly elevated values were noted ($p>0.05$).

In advanced IDA, cellular immunity is adversely affected, and bactericidal functions decrease. IDA increases oxidative stress [31,39]. In our cases increase in HSP70 levels during post-IDA therapy might be due to oxidative stress induced by the administration of oral iron supplements. Post-treatment hyperthermia might also explain this rise in HSP70 levels. The highest post-IDA treatment HSP70 levels might be attributed to higher values found in newly formed erythrocytes. With the maturation of erythrocytes, levels of HSP70 decrease. During the maturation of reticulocytes, HSP70 might be influential in the elimination of superfluous proteins [40].

Secretion of HSP70 is stimulated by ghrelin [10,31]. In our cases diagnosed as IDA, the detection of higher HSP70 values in comparison to the control group despite lower ghrelin levels contradicts this phenomenon (Table **3**). HSP70 levels measured during the pretreatment period of cases diagnosed as IDA did not differ significantly from corresponding values obtained during the post-treatment period (peak level) and from the control group (trough levels) (Table **3**).

HSP70, whose release is induced by ghrelin, has been observedly to rise to higher concentrations in stressful conditions, but its levels have not demonstrated significant fluctuations in the control group and also during the pre-and post-treatment period of the cases diagnosed as IDA. Our literature survey has demonstrated that the alterations in HSP70 levels have not been analyzed. In our study, lower HSP70 concentrations were thought to be related to suppressed ghrelin levels and lower body temperature. It is difficult to expound on the underlying pathogenetic mechanism. We have not revealed any impact of anemia and its treatment on the level of HSP70 in our cases which might be attributed to the gradual development of IDA and prolongation of treatment. Gradually employed oral treatment lasting for 3 months which might not deteriorate normal physiologic mechanisms without triggering any stressful cascade, could not possibly influence HSP 70 levels.

In our study, IDA was found to increase the predisposition to hypothermia (Table **3**). Post-treatment HSP70 levels increased, though not significantly, when compared with both pretreatment levels, and especially the control group. The level of HSP70 might increase predominantly with parenteral (intramuscular, intravenous) iron therapy [41], which leads to numerous unfavorable changes.

The importance of the obestatin level in IDA has not been studied in the literature. Any difference in body weights of our cases diagnosed as IDA and the control group was not detected. However, a decrease in ghrelin and an increase in obestatin levels can suppress weight gain and appetite. Obestatin can lead to the intake of diverse kinds of foods. In our study, the lack of statistically significant difference between ghrelin levels and obestatin in cases diagnosed as IDA might be attributed to the scarcity of cases. (Table **3**, Figure **2**). Groups of patients with IDA comprising a higher number of pica cases should be studied. Ratios of obestatin/acyl ghrelin and obestatin/des-acyl ghrelin were found to be higher relative to corresponding estimates in the non-pica group. Lower obestatin/acyl ghrelin and obestatin/des-acyl ghrelin ratios might induce pica disorder.

Ghrelin levels were estimated to be relatively higher in the IDA-Pica Group. Pica has been reported to be associated with severe IDA in up to half of patients. Acyl and des-acyl ghrelin and HSP 70 levels were higher in the Pica group compared to the Non-Pica group, and there was no statistically significant difference between them. Obestatin ($p>0.05$) and obestatin/acyl ghrelin (<0.05) levels were lower in the Pica group. A scarce number of cases (n=9) might obscure intergroup differences. A statistically significant difference between obestatin/acyl ghrelin and

obestatin/des-acyl ghrelin ratios has much more significance.

CONCLUSIONS

Levels of HSP 70, whose release is induced by ghrelin, have risen to higher concentrations in stressful conditions and have not demonstrated significant fluctuations in the control group and during the pre-and post-treatment period of the cases diagnosed as IDA. This phenomenon was conceived to be related to lower levels of ghrelin and body temperature. Lower ghrelin and higher obestatin levels might contribute to the development of IDA. Higher obestatin and obestatin/acyl ghrelin ratios may prevent the development of pica disorder. These findings observed should be substantiated with larger-scale studies. The etiology of pica, the purposive consumption of non-food substances, is not understood, despite its ubiquity among gravidae. The nature of the relationship between pica and IDA merits further investigation.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

ACKNOWLEDGMENTS

All authors contributed to the study design and revised the manuscript, which was drafted by Dr. Akarsu and Dr. Taner Kasar. All authors approved the final version submitted for publication.

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Received on 23-01-2023 Accepted on 17-02-2023 Published on 24-03-2023

https://doi.org/10.6000/1929-4247.2023.12.01.1