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RESEARCH ARTICLE

Serum level of Leptin and Ghrelin in Juvenile Rheumatoid Arthritis and their correlation to disease activity and body mass index

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Abstract

Aim of the work: This study was designed to investigate the serum level of Leptin and Ghrelin in Juvenile Rheumatoid Arthritis (JRA) and its correlation to disease activity and body mass index (BMI)

Patients and methods: This study included sixty JRA patients (38 females and 22 males) and forty matched healthy controls (26 females and 14 males). levels of ghrelin and leptin were measured by ELISA and correlate these levels with Juvenile Arthritis Disease Activity Score (JADAS) and patients BMI. Complete blood count (CBC), Erythrocyte sedimentation rate (ESR) mm/1st h, Serum C- reactive protein (CRP) and interleukin 6 were measured.

Results: Significant increase of serum level of Leptin and IL-6 and significant decrease of serum level of ghrelin in patients group as compared with control group. Significant increase in serum level of leptin in active group as compared with inactive group, while there was no significant change in serum level of ghrelin in active as compared with inactive patients. Significant positive correlations were found between ghrelin and leptin serum levels and patients' BMI.

Conclusion: leptin and Ghrelin may be responsible for weight loss in JIA, and may have a role in the pathogenesis of the disease.

INTRODUCTION

Juvenile idiopathic arthritis (JIA), also known as juvenile rheumatoid arthritis (JRA), is the most common form of arthritis in children and adolescents [1]. JIA is an autoimmune, non-infective, inflammatory joint disease of more than 6 weeks duration in children less than 16 years of age. The disease commonly occurs in children from the ages of 7 to 12, but it may occur in adolescents as old as 15 years of age, as well as in infants [2]. It is a subset of arthritis seen in childhood affects between 8 and 150 of every 100,1000 children. It may be transient and self-limited or chronic. It differs significantly from arthritis commonly seen in adults (osteoarthritis, rheumatoid arthritis), and aetiopathology is similar to rheumatoid arthritis but with less marked cartilage erosion, and joint instability and absent rheumatoid factor [2].

Leptin is non-glycosylated cytokine-like hormone synthesized by white adipose tissue. Leptin and its receptors (Ob-R) share structural and functional similarities with cytokines of IL-6 family and their receptors. Lepten has an important role in the regulation of body weight by inhibiting food intake and stimulating energy expenditure [3].

Peripheral functions of leptin include regulation of endocrine function, reproduction and immunity[3]. During acute inflammation, pro-inflammatory cytokines increase circulating leptin concentration, and leptin, in turn, potentiates cytokine release from monocytes/macrophages [4].

Ghrelin is a novel growth hormone (GH) releasing peptide, isolated from the stomach, that is identified as the endogenous ligand for GH secretagogue receptor [5]. It has been showed that ghrelin produces a positive energy balance by stimulating food intake [6] and decreasing fat utilization through a GH-independent mechanism. It also antagonizes leptin through the activation of the hypothalamic neuropeptide Y/Y1 receptor pathway [7].

The aim of this study was to assess the serum level of leptin and Ghrelin and their relationship with disease activity parameters and body mass index (BMI) in Egyptian patients with Juvenile Rheumatoid Arthritis (JRA).

Subject and Methods:

1- Study subjects

Sixty Juvenile rheumatoid arthritis patients (38 females and 22 males) aged from 6 to 15 years and have disease duration ranging from 6 to 60 months were enrolled in this study. They were selected from the outpatient clinic of the Rheumatology and Rehabilitation and Pediatric Departments, Tanta University Hospitals, Egypt. They fulfilled The International League of Associations for Rheumatology (ILAR) criteria for JRA. [8] All patients were under disease modifying anti-rheumatic drugs (DMARDs) and/or NSAIDs. In addition, forty healthy volunteers (26 females and 14 males) matched in age were included in this study.

Patients having diabetes mellitus, endocrinal disease (Cushing syndrome, thyroid diseases), patients taking corticosteroid during the last 6 months and those having any malignancy were excluded from the study. Informed consent was obtained from all children' s parents before enrollments and the study approved by the local ethical committee of faculty of medicine, Tanta University. All cases included in this study were subjected to detailed history taking and rheumatologic and systemic examination.

-BMI was calculated as weight [in kilograms] divided by squared height [in meters] (kg/m^2). BMI below 5th percentile reflects underweight, BMI between 5th and 84th percentile reflects normal weight, BMI between 85th percentile and 94th percentile reflects at risk for overweight and BMI above 95th percentile reflects overweight [9].

- Assessment of JIA activity (in patients): by Juvenile Arthritis Disease Activity Score (JADAS) [10, 11].

2- Sampling:

7ml of venous blood were withdrawn from patients and control subjects under complete aseptic precautions 1.6 ml blood was transferred into a vacutainer tube containing 0.4ml sodium citrate for determination of ESR, 1 ml was placed in EDTA containing vacutainer tube for complete blood count and the rest of the blood was delivered in a plain glass tube, allowed to clot at room temperature, centrifuged at 2000 rpm for 10 minutes and serum was separated. CRP were determined immediately and aliquots of the rest of the serum were stored at -70°c till the time of assay of other laboratory tests.

3- laboratory investigations including:

1. Complete blood count (CBC).
2.Erythrocyte sedimentation rate (ESR) mm/1st h was determined by Westergreen accordingto Dacie and Lemis [12], ESR > 28 mm/1st h was considered as criteria of disease activity
3.Serum C-reactive protein (CRP) was determined by semi-quantitative latex agglutination using AVITEXCRPLATEX kit (Omega Diagnostics, Scotland, UK) according to Hind and Pepys [13]. CRP value > 6mg/L was considered positive.
4.Serum leptin concentration was measured using Human Leptin ELISA kit supplied by Diagnostic System Laboratories, INC, USA [14].

5. Serum total ghrelin was measured using commercially available total ghrelin ELISA kit supplied by DSL-10-33700, INC. USA [15].
6.Serum IL-6 was measured by specific, commercially available, enzyme linked (ELISA) assay kits (Quantikin e, R&D Systems Inc, USA) in accordance with the manufacturer's instructions and analysed with an ELISA re ader at 492 nm [16].

Statistical analysis

Statistical analyses were performed with a Statistical Package for the Social Sciences (SPSS version 8.0 Package (SPSS, USA)). Values were expressed as mean ±SD . Comparisons of parameters between the two groups were made by nonparametric t-test The relationships between the different variables were analyzed using Spearman non-parametric correlation coefficient. A P value ≤ 0.05 was considered significant.

Results:

Table (1): Shows the demographic characteristics and laboratory findings of JIA patients and control groups. The mean ± SD of age were 11.2±2.4 and 12.1±1.9 years in JIA and control groups respectively, females predominate in both groups, the type of JIA onset were 19 patients with systemic onset, 25 patients polyarticular and 16 patients oligoarticular. Disease duration, Mean ±SD was 36±12.

There is no significant difference between both groups as regard ESR and CRP. Also, the table showed significant increase of serum level of Leptin and IL-6 and significant decrease of serum level of ghrelin but no significant change in serum level of ghrelin in patients group as compared with control group. There are also significant decrease in BMI in patient group as compared to control group.

Table (2): Shows serum level of leptin, ghrelin in active and inactive groups. It shows that significant increase in serum level of leptin in active group as compared with inactive group, while there is no change in serum level of ghrillin in active as compared with inactive patients.

Table (3): Shows significant correlation of serum level of leptin and ghrelin with BMI.

Table (1). Demographic data and laboratory characteristics of JIA patients and control subjects

Parameter	JIA patients n=60 Mean ±SD	Control group n=40 Mean ±SD	P
Age(years)	11.2±2.4	12.1±1.9	NS
Sex Males Females	 22 38	 14 26	NS
Pattern of JIA: Systemic onset Polyarticular Oligoarticular	 19 25 16	-	
Duration of disease (months)	36±12	-	
ESR	11.16±11.01	9.70±3.38	NS
CRP	6.10±11.12	5.9±13.13	NS
Leptin (ng/ml)	25.0 ± 9.6	20.5± 6.9	<0.05

Ghrelin (pg/ml)	28.5 ±9.1	121.0 ±9.7	<0.001
IL-6 (pg/ml)	101± 2.7	2.5 ± 3.0	<0.001
BMI(kg/m2)	15.14±9.11	20.14±2.11	<0.001

Table (2). Serum levels of leptin and ghrelin in active and inactive patients

	Active patients n=36	Inactive patients n=24	P
Leptin (ng/ml)	25.1 ± 8.5	23.8 ± 4.4	<0.001
Ghrelin (pg/ml)	27.6 ±8.7	28.1 ±5.6	NS

Table (3). Correlation of serum levels of leptin and ghrelin with BMI

	r	P
Leptin	0.256	<0.05
Ghrelin	0.243	<0.05

Discussion:

Leptin is considered a new pro-inflammatory adipocyte derived factor that operates in the cytokine network by linking immune and inflammatory processes to the neuroendocrine. It has been shown that acute inflammation and pro-inflammatory cytokines IL-6 positively regulate leptin expression in adipose tissue and circulating blood. Also, Leptin and its receptors (Ob-R) share structural and functional similarities with cytokines of IL-6 family and their receptors [3]. There is a positive correlation between BMI and circulating leptin concentration has been previously reported in patients with growth delay [17] or with JIA [18].

Our study showed that significant increased level of leptin in JIA group as compared to control group (<0.05) and significant positive correlation with disease activity (<0.001) and BMI (<0.05). The increase in leptin production during infection and inflammation strongly suggests that leptin is a part of the cytokine cascade, which orchestrates the innate immune response and host defense mechanisms. Also, leptin may promote inflammatory response via production of nitric oxide synthase type 2 and it potentiates cytokine release from monocytes/macrophages [19]. In addition, leptin stimulates T-cell mediated immunity and is able to induce the proliferation and differentiation of haemopoietic cells [20].

Our results are in agreement with Agata et al. [21] who reported that leptin concentration in sera of JIA children was higher than in healthy children, also reported that in JIA children with high disease activity leptin concentration was higher than in children with low disease activity, but without statistical significance. However, they found a positive correlation between serum leptin concentration and BMI of their patients and concluded that high leptin concentration in JIA children and its correlation with BMI could indicate leptin' s role in body mass regulation in the course of the chronic inflammatory process.

Other researchers also reported higher level of leptin in adult Egyptian patients with JIA than in controls [22]. Abdalla et al. reported a positive correlation between leptin level and BMI of Egyptian adults with JIA but no correlation was found between leptin and disease activity [23]. Winiarska et al. reported no significant difference in serum level of leptin between JIApatients and controls [24]. Other studies demonstrated that plasma leptin in JIA patients was significantly lower than in controls [25]. Also previous researchers mentioned that circulating leptin concentration was not related to the activity of the disease but significantly related to BMI of JIA patients either

white children [1] or Egyptian adults [18]. Our results also come in agreement with the work of other studies that reported a positive correlation between BMI of RA patients and serum levels of leptin [26].

Ghrelin is a powerful, endogenous orexigenic peptide and it has anti-inflammatory effects. It has been reported that ghrelin down-regulates pro-inflammatory cytokines, including interleukin (IL)-1 beta and TNF-α [27]. Li et al. demonstrated that ghrelin attenuated TNF alpha induced nuclear translocation $NF\text{-}_KB$, indicating that blockade for activation of the transcription factor $NF\text{-}_KB$ could be a potential mechanism whereby ghrelin modulates inflammatory responses [28]. Other studies show that ghrelin produces a positive energy balance by stimulating food intake [29] and decreasing fat utilization through a GH-independent mechanism. It also antagonizes leptin through the activation of the hypothalamic neuropeptide Y/Y1 receptor pathway [30]. Interestingly, ghrelin may promote that ghrelin may play an important role in the regulation of metabolic balance in inflammatory diseases such as RA [31].

As regard the serum level of ghrelin, the result of our study demonstrated significantly decreased serum level of circulating ghrelin in patients with JIA compared to controls but there was no significant change in serum level of ghrelin in active as compared with inactive patients,. Also, the results showed that significant correlation with BMI. In agreement with our results, Karagiozoglou et al. reported decreased ghrelin level in patients with juvenile idiopathic arthritis, and also showed a significant relationship between ghrelin and disease activity, but they did not find a significant relationship between ghrelin and nutritional status, [32] while El-Eshmawy et al. reported negative correlation between serum ghrelin and BMI of Egyptian adolescents [17].

Our data indicate that ghrelin may play an important role in ameliorating pathologic inflammatory states.Sanem Eren et al. demonstrated no significant difference between JIA patients and controls as regards serum level of ghrelin[25] and Koca et al.[27] did not find significant relation between serum level of ghrelin and disease activity in patients with JIA. Zhao et al strongly suggested that ghrelin may be a proinflammatory peptide in the colon and it may participate in the pathophysiology of colonic inflammation by inducing protein kinase (PKC)-dependent NF-kappa B activation and IL-8 production at the colonocyte level [33]. Ghrelin also inhibited endotoxin-induced systemic cytokine production in vivo. These findings may help to explain the beneficial effects of ghrelin administration in various pathological states associated with inflammation.

Conclusion and recommendations:

Ghrelin and leptin may be responsible for weight loss in JIA, and may have a role in the pathogenesis of the disease. Further studies must be done on larger scales to study ghrelin and leptin as potential targets of new therapeutic strategies to correct anorexia and associated weight loss in JIA patients.

Conflict of interest:
None declared.

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