

Serum leptin level in rheumatoid arthritis and its relationship with disease activity

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Abstract

Objectives: To evaluate serum leptin levels in rheumatoid arthritis (RA) patients and to investigate its correlation with parameters of disease activity including DAS-28, C- reactive protein (CRP) and ESR.

Materials and Methods: This study included 60 rheumatoid arthritis patients attended Ibn-Sina and Al-Salam Teaching Hospitals during the period from December 2013 to June 2014. The study also included 30 apparently healthy individuals as a control group who were age, gender, and merely body mass index matched to rheumatoid arthritis patients. Full clinical assessment of the patients was conducted by consultant rheumatologists/physicians. A Questionnaire Form was completed and DAS-28 was calculated. Five milliliters of blood were collected from patients and controls, then centrifuged and the serum was separated and frozen as aliquots at -20°C until use. The sera were tested by enzyme linked immune-sorbent assay (ELISA) for leptin.

Results: Serum leptin levels in RA patients in comparison to the control group were within normal range. Its value varied ranging between 0.7 to 7.5 ng/ml (in patients), and mean $\pm$ standard deviation in RA patients 2.1 ± 4.2 and in controls 3.13 ± 2.30 . In RA patients, there was no correlations between serum leptin levels and DAS-28, CRP, ESR. Serum leptin levels positively correlated with body mass index (BMI) in both patient and control groups.

Conclusions: Circulating leptin level does not seem to reflect disease activity in rheumatoid arthritis since there is no correlation between its serum levels and parameters of disease activity namely DAS -28, CRP, ESR.

Key words: leptin, DAS-28, BMI, rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is a progressive inflammatory autoimmune disease with articular and systemic manifestations [1]. It affects about 0.5 % to 1% of population worldwide with female to male ratio of 3:1[2]. Recent studies demonstrated that cytokines secreted by white adipose tissue (WAT) called "adipo-kines", have an important role in the pathophysiology of RA [3]. Some of these adipokines are hormones-like factor such as leptin and adiponectin [4]. Studies showed that there is a close relationship between serum level of leptin and the aggressive course of RA [5,6]. Data about the role of leptin in RA have been reported that serum leptin concentration could be similar [6,7,8], lower [9] or higher [10] than that of apparently healthy control. The DAS -28 is a system developed and validated by the European League Against Rheumatism (EULAR) to measure the progress and improvement. Twenty-eight" describes the number of different joints mainly proximal interphalangeal joints (10 joints), metacarpophalangeal joints (10), wrists (2), elbows (2), shoulders (2), knees (2), ESR is also measured [11]. The affected person makes a global subjective assessment of disease activity during the preceding 7 days on a scale between 0 and 100, where 0 is "no activity" and 100 is "highest activity and then all of these are included in a special equation and measured by a special program in the computer [12]. From this, the disease activity of the affected person can be classified into two groups depends on DAS -28 score, inactive core is ≤ 3.2 , while the active score is > 5.1 [12].

Materials and Methods

The present study was conducted in the Diagnostic Laboratory at the Department of Microbiology,

College of Medicine, University of Mosul. The data of the present study were collected during the period from December 2013 to June 2014. A total of 60 Iraqi RA patients, fulfilled the American Rheumatism Association criteria (ARC) for diagnosis of RA were enrolled in the current study. A special Questionnaire Form of relevant clinical data was completed for each patient. The age of patients ranged from 20 to 79 years. Some of these patients were attending the Outpatient Clinics and others were inpatients admitted to the Department of Rheumatology in Ibn-Sina and Al Salam Teaching Hospitals. DAS -28 was calculated to assess the severity of the disease by a special program in the computer. BMI was also calculated to each patient and control. ESR, C-reactive protein (CRP), were collected from patients' records. A total of 30 apparently healthy individuals were enrolled in the present study as a patient – matched control group from age, gender, and BMI points of view. They were medical staff or blood donors. They have no history or clinical evidence of RA or any other chronic or rheumatic disease.

Sample collection, handling and storage

Under a septic conditions five milliliters of blood were collected from each patient and control individuals. The blood sample was then centrifuged and the sera separated and kept frozen at -20°C .

ELISA test for leptin

The detection of leptin was performed by an in vitro sandwich Enzyme- Linked Immunosorbent Assay kit manufactured by DIA source Belgium, France. The microtiter plate was coated with a monoclonal antibody 1(MAb1) directed against distinct epitopes of human leptin. ELISA test was done according to manufacturer's procedure.

Statistical analysis

The data were analyzed statistically by using tables , pie and bar charts. Standard statistical methods were used to describe the results of the study : mean , standard deviation (SD), standard error (SE), number and percentage. Correlation analysis was performed using Pearson's correlation coefficient factor (r) Excel 2007 program was used [21].

Results

The higher frequencies of RA were observed at fifth and sixth decade at a frequency of (15%, 26%) respectively (Figure1). Higher incidence was noticed among the female (73.3 %) than in male (26.6); and male to female ratio was 1:2.75 ratio (Figure 2). The average of the patient's age was between 20-79 years , with a mean $\pm$ SD of 49.21 ± 13.12 years. The age of normal control group ranged between 25- 75 years, with a mean $\pm$ SD of 50.1 ± 12.9 years.

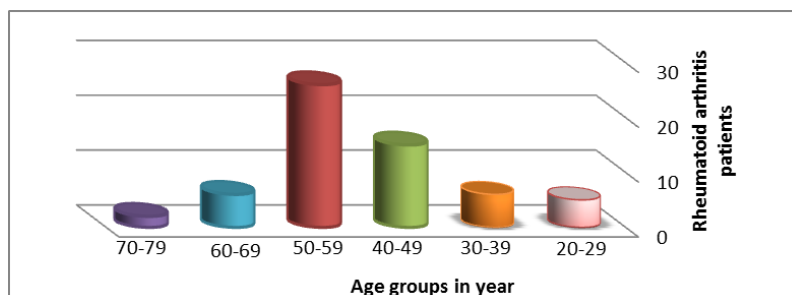


Figure 1: Age distribution among RA patients

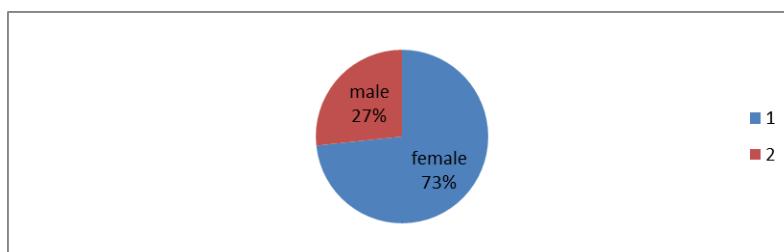


Figure 2: Male to female ratio of RA

In RA patients serum leptin level was found within normal ranges (Table1). Its value varied ranging between 0.7 to 7.5 pg/ml with a mean $\pm$ SD was 4.28 ± 2.14 , 3.13 ± 2.30 in RA and control group

respectively Figure3. There was no a significant difference between serum concentration of leptin among RA patients and healthy control group, (Figure 3).

Table 1: The levels of serum leptin among patients and control groups

Healthy controls	No.	Mean serum leptin level (ng/ml)
	30	4.28 (ng/ml)
RA patients	60	3.13 (ng/ml)
P	< 0.1	

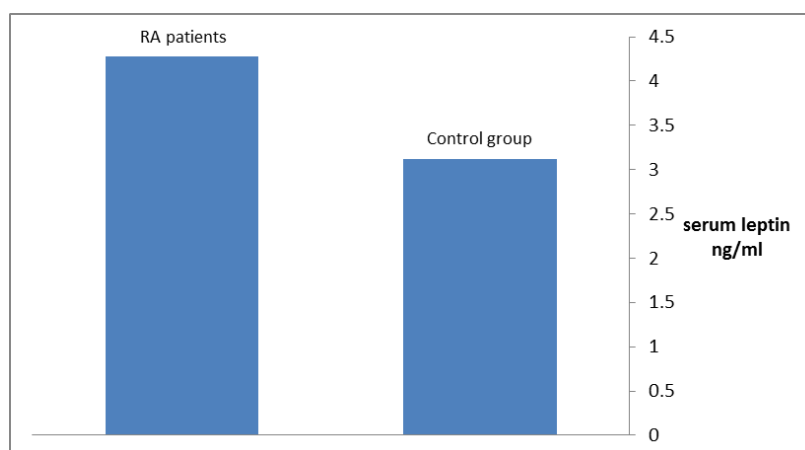


Figure 3: Difference between mean values of leptin in RA and control groups

Relationship between serum leptin, disease activity parameters and BMI

DAS -28 of the patients was calculated for each patients calculated to assess the severity of the disease by a special program in the computer. It depends on number of tender joints, swollen joints, patients global assessments of disease and ESR results. Its value varied ranging between 2.55 to 7.46

with a mean $\pm$ SD was 5.02 ± 0.88 . In RA patients there was no correlation between serum leptin level and CRP, ESR and DAS-28.(Figure 4,5,6) . Body mass index was calculated for each patient and control. Its value was varied with a range from 15.89 to 29.98. There was a strong positive correlation between BMI in both control and patients group, and leptin concentration at $r = 0.9$ (Figure 7).

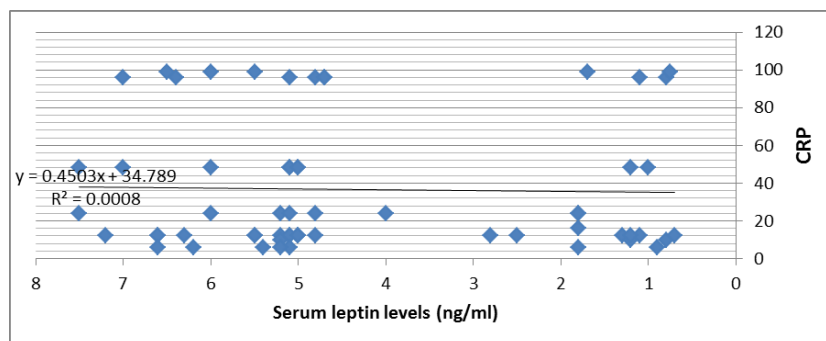


Figure 4: Relationship between serum leptin and CRP in RA patients

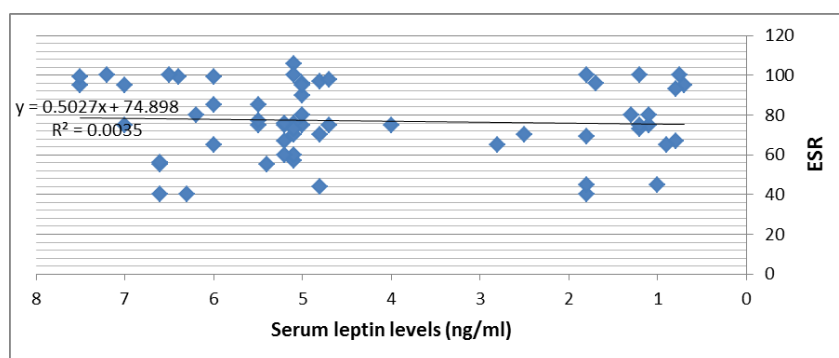


Figure 5: Relationship between serum leptin and ESR in RA patients

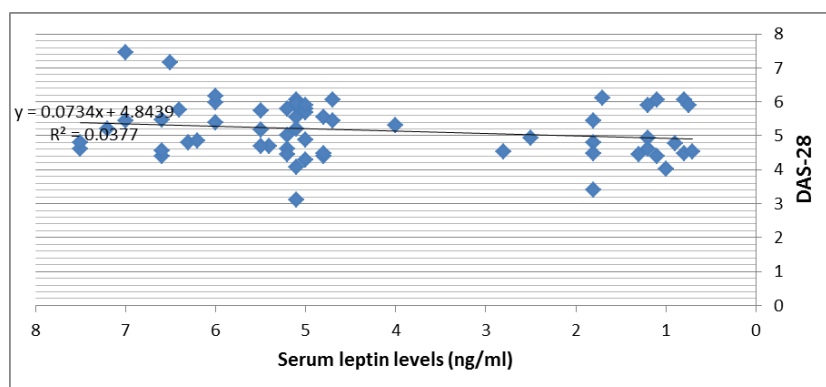


Figure 6: Relationship between serum leptin and DAS -28 in RA patients

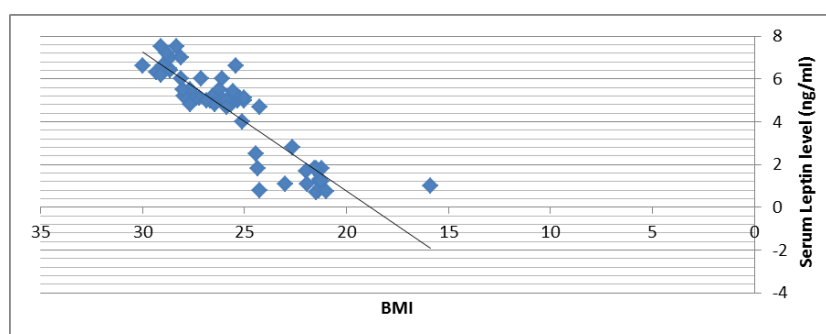


Figure 7: The relationship between BMI and serum leptin levels in RA patients

Discussion

Rheumatoid arthritis (RA) is a world-wide disease with a wide variation in the incidence, prevalence, severity and clinical manifestations [13].

In the present study, the predominant age groups affected with RA were in the fifth and the sixth decade of life. This differs from what have been reported from other countries in Europe, in which the predominant age groups were in the fourth and fifth decade [14]. This difference in age incidence is probably due to genetic, environmental or other precipitating factors among RA patients in Mosul city.

The results denoted a high prevalence of RA among women than men, which may be due to the hormonal differences between them and in turn, their effects on the immune response (IRs) [15]. The female to male ratio in this study was 2.75:1 which is nearly comparable to most other studies from Iraq in which the ratio ranged between 2.7:1 to 3:1 [2]. Recent studies on serum leptin levels in RA patients showed that controversial results, as it could be similar, lower or even higher than its level in control group [6,7,8,9,10]. According to this study serum leptin level in RA patients was similar to healthy control individuals. This is in agreement with three earlier studies done in Europe by Targonska S. B., 2008, Hizmetli S., 2007, Popa C., 2005. The lack of difference in between RA patients and control group was related to many factors such as the effects of medications used for treatment, the difference in body mass indices of patients with RA and control group, and the most important is the effect of chronic inflammation on serum leptin level. Chronic inflammation as that occurs in RA, down regulate leptin circulating level towards normal value or even lower [16]. As shown in a study of Bruun and Coworkers [16] which considered that long-term stimulation of adipose tissue by TNF- α or IL-1 inhibits leptin and leptin mRNA production. On contrary to a study done by Sarraf et al. [17] who mentioned that in the process of chronic inflammatory diseases, pro-inflammatory cytokines (IL-1 and TNF- α) increase obese gene expression and

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leptin secretion. The reasons for this discrepancy is still not obvious whether the effect of weight change or it is rather a cause or a consequence of pathology in RA [18].

In this work, no significant correlation was found between the serum leptin level and disease activity parameters such as ESR, CRP and DAS -28 score. This is in keeping with the findings of other studies [6, 20] which suggesting that leptin does not seem to reflect disease activity of RA. Wisolwska and Associates [20] done a study showed that there was no correlation between serum leptin level and age of RA patients, stage of disease, number of painful and swollen joints, duration of morning stiffness, disease duration as well as value of DAS -28 and presence of rheumatoid nodules. On other hand, Seven and Colleagues [19] found that RA patients with moderate disease activity (DAS > 2.7) had significantly higher leptin levels than those with low disease activity (DAS < 2.7). In addition, Yoshino and associates [3] reported that serum levels of resistin and leptin were positively associated with the CRP level in patients with rheumatoid arthritis, suggesting that these adipokines may act as pro-inflammatory cytokines in this disease.

In the present study, a significant positive correlation was found between serum leptin levels and BMI of the RA patients and controls. This result is in agreement with the work of other studies in America and Europe [8]. Targojska-Stdpniak and Colleagues [10] found that leptin concentration correlated positively with BMI in women with RA, but not in men. However, Popa and coworkers [7] found that in RA patients, plasma leptin concentration did not correlate with BMI. Also, Seven and Colleagues [19] reported no correlation between leptin and BMI. They suggested that regulation of leptinemia is complex and that weight is not the only major regulator.

In conclusion, circulating leptin levels did not seem to reflect disease activity in this study. Since many aspects of the biology of leptin remain unclear, further researches on leptin hormone in relation to other hormonal changes in rheumatoid arthritis patients are recommended.

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قياس مستوى اللبتين في مصل مرضى التهاب المفاصل الرثواني وعلاقته مع فعالية المرض

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الملخص

الاهداف:- لتقييم مستوى اللبتين في مصل مرضى التهاب المفاصل الرثواني وإيجاد علاقته مع معلمات نشاط المرض وهي تشمل مقياس مستوى نشاط المرض - ESR, 28 ومعامل التفاعل ث.

عينات الدراسة وطرائق المرض:- هذه الدراسة شملت 60 مريضاً مشخصين بالتهاب المفاصل الرثواني والذين راجعوا مستشفى ابن سينا التعليمي و مستشفى السلام التعليمي وخلال الفترة من كانون الاول 2013 والى حزيران 2014. كذلك شملت هذه الدراسة 30 شخصاً سليماً والذين كانوا مطابقين في اعمارهم و اجناسهم و اوزانهم تقريباً للمرضى , وبعد اخذ معلومات كاملة من كل مريض وفحصهم سريريا تم ملئ استمارة الاستطلاع الخاصة بهذه الدراسة ثم تم اخذ 5 مل من الدم من كل من المرضى والاصحاء, تم استخلاص مصل الدم بعملية الطرد المركزي ووضع كل عينة في 5 انابيب ابيندروف ومعلمين بأسماء المرضى ثم تجميدهم لحين تم اجراء الفحوصات عليهم.

النتائج:- مستوى اللبتين في مصل المرضى كان ضمن الحدود الطبيعية مقارنة مع الاصحاء وتراوحته نسبته بين 0.7-7.5 نانوغم/مل ومتوسطه 4.2 ± 2.1 نانوغم/مل عند المرضى وكان متوسطه عند الاصحاء 3.13 ± 2.30 نانوغم/مل.

لا توجد علاقة بين مستويات اللبتين ومعلمات نشاط المرض وهي تشمل مقياس مستوى نشاط المرض - ESR, 28 ومعامل التفاعل ث.

الاستنتاجات:- لا يعكس مستوى اللبتين في مصل مرضى التهاب المفاصل الرثواني فعالية المرض ويتناسب طردياً مع معامل كتلة الجسم.

الكلمات الدالة:- اللبتين, معلمات نشاط المرض, معامل كتلة الجسم, التهاب المفاصل الرثواني