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Brief Report

Peripheral blood Leptin and Resistin levels as clinical activity biomarkers in Mexican Rheumatoid Arthritis patients

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ABSTRACT

Objective: To evaluate the association between the clinical activity of RA patients and serum adipocytokines (Leptin, Adiponectin and Resistin) and inflammatory cytokines.
Methods: All RA patients fulfilled ACR 1987 criteria and were treated with DMARDs. Adipocytokine and inflammatory cytokine levels were evaluated using ELISA.
Results: 121 patients were included in the study. Stratifying according to DAS28 (low, moderate and high activity), there were significant differences for Leptin, Resistin, IL-6 and IL-17, however, no differences were seen for Adiponectin, TNFα or IL-1β. Clinical activity positively correlated with Leptin, Resistin, IL-17 and IL-6 levels, but not with Adiponectin, TNFα or IL-1β. Adiponectin levels negatively correlated with TNFα and positively correlated with IL-1β. IL-1β positively correlated with IL-6 and negatively correlated with TNFα and IL-17.
Conclusion: Circulating Leptin, Resistin, IL-6 and IL-17 levels positively correlate with RA clinical activity in a manner independent of the subject's BMI. Complex relationships between inflammatory cytokines were observed in RA patients suggesting that other metabolic or inflammatory factors could be involved.

Niveles en sangre periférica de Leptina y Resistina como biomarcadores de la actividad clínica de pacientes mexicanos con Artritis reumatoide

Palabras clave:
Artrtitis reumatoide
Leptina
Resistina
Adiponectina
Citocinas pro-inflamatorias
Actividad clínica

RESUMEN

Objetivo: Evaluar la asociación entre la actividad clínica de pacientes con Artritis reumatoide y adipocitocinas séricas (Leptina, Adiponectina y Resistina), citocinas inflamatorias (TNFα, IL-1β, IL-6, IFNγ e IL-17A).
Métodos: Se seleccionaron pacientes con AR (ACR 1987) tratados con FARMEs. Los niveles de adipocitocinas y citocinas inflamatorias fueron evaluados por ELISA.
Resultados: 121 pacientes se incluyeron en el estudio. La actividad clínica correlacionó positivamente con Leptina, Resistina, IL-6 e IL-17 pero no para Adiponectina, TNFα o IL-1β. Los niveles de Adiponectina se asociaron negativamente con TNFα y positivamente con IL-1β. Por su parte, IL-1β se asoció de manera positiva con IL-6 y negativamente con TNFα e IL-17.
Conclusión: Los niveles circulantes de Leptina, Resistina, IL-6 e IL-17 se asociaron de manera positiva con la actividad clínica de pacientes con AR, independientemente del índice de masa corporal (IMC). Asimismo, en los pacientes con AR se observaron asociaciones complejas entre las adipocitocinas y citocinas, sugiriendo que otros factores tanto metabólicos como inflamatorios pudieran estar involucrados.

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Introduction

In recent years there has been a lot of interest in studying the relationship between adipocytokines and disease activity in Rheumatoid Arthritis (RA), however results are heterogeneous. Several studies have observed higher peripheral blood concentrations of Leptin in RA than in healthy subjects,[1] as well as an association with some inflammatory markers[2] and disease activity.[3] Additionally, other studies have observed an association with insulin-resistance[4] and endothelial activation[5] but not with radiographic progression.[6] In a two-year-follow up study of patients with RA, a positive correlation between Leptin and IL-17 and a decrease in DAS28 was seen in patients treated with DMARDs.[2] Similarly, high levels of Adiponectin have also been reported in RA[1] and associated with radiographic progression.[6]

Resistin has been also studied in RA, with heterogeneous findings; some studies reported an association with inflammatory markers[7] and clinical activity.[8] Baseline Leptin levels predict clinical activity and response to DMARD treatment in non-overweigh/non-obese RA patients,[9] suggesting both a role for Leptin in the systemic abnormalities seen during the course of the disease, as well as the influence of adiposity on the RA inflammatory process. However, the association between adipocytokines with inflammatory cytokine levels is poorly understood. Therefore, the objective of this study is to describe the relationship between Leptin, Adiponectin and Resistin with the disease activity of RA patients and the levels of several inflammatory cytokines.

Material and methods

Patient selection

Patients were recruited at the Rheumatology clinic of the Hospital General de Cuernavaca in Morelos, Mexico between 2007 and 2009. The study protocol was reviewed and approved by the Hospital ethics' committee. All patients followed a similar treatment strategy: methotrexate (7.5–15 mg/week) in combination with cloroquine 150 mg/d and prednisone ≤10 mg/day. *Inclusion criteria:* Patients included were seen consecutively and had Rheumatoid Arthritis, classified according to the American College of Rheumatology 1987 criteria[10]; their ages ranged from 18 to 75 years; signed informed consent was obtained from all of the patients and they all had a complete clinical file at the hospital. Disease activity was measured using DAS28 based on the number of swollen and tender peripheral joints, the patients' overall assessment by visual analog scale (VAS) and the erythrocyte sedimentation rate (ESR). The patients underwent a clinical evaluation performed by a single rheumatologist; venous blood samples were withdrawn afterwards and plasma samples were separated on the same day of collection and frozen at −75 °C. All patients received combination treatment with DMARDs (mainly methotrexate and cloroquine) and, in some cases, 10 mg or less per day of prednisone. NSAID and analgesics were prescribed on demand. No patients were treated with biologics due to health coverage limitations. For the comparative analysis, patients were stratified according to DAS28 into the following groups: low disease activity (DAS28 ≤3.5), moderate activity (DAS28 >3.5 to ≤5.1) and high disease activity (DAS28 >5.1).

Quantification of adipocytokine and cytokine blood levels

Leptin, Adiponectin, Resistin, IL-1β, IL-6, IL-17 and TNFα levels were determined from venous blood plasma by optimized ELISA employing specific monoclonal antibodies (Santa Cruz Biotechnology, eBioscience). Calibrated curves were prepared in each plate employing a recombinant human standard for each metabolite (Peprotech Inc.). All essays were performed by in triplicate for each sample. Calibration curves fitted a linear regression with a correlation higher than 0.85 and a p value <0.05%.

Statistical analysis

Descriptive statistics were employed and results were expressed as means and standard deviations. A one way ANOVA (Kruskal–Wallis test) was performed to compare DAS28-patients subgroups. The comparison between groups was done using the Mann–Whitney test and correlation between variables was determined using Spearman's test (PRISM v.6.0), controlling for gender, age, years since onset of disease, BMI and ESR (STATA v.13). The level of statistical significance was set at 0.05%.

Results

121 patients with RA were included in the study. It is worth recognizing there were differences in the number of patients for each activity subgroup, with only 22 patient in the low activity group, while 56 patients had moderate activity (Table 1). However, the three subgroups were similar in gender, age, BMI, time since onset of disease and RF or anti-CCP antibody titers. Although most patients were overweight, no differences were seen between the activity subgroups.

There was a significant difference in Leptin, Resistin, IL-6 and IL-17 levels when comparing between activity subgroups using one-way ANOVA (Table 1). In the particular case of Leptin, when comparing DAS28 subgroups, all showed mutual statistical differences ($p < 0.001$), suggesting a positive relation with the increase in the DAS28 score. In a similar manner, a significant mean difference for Resistin levels was observed by comparing the low vs. high activity subgroups ($p < 0.0007$); however, this was not the case when comparing the moderate vs. high activity subgroups ($p = 0.080$) or when comparing the low vs. moderate activity subgroups ($p = 0.107$). In contrast, Adiponectin did not shown differences between clinical activity subgroups. For its part, the low activity subgroup showed lower IL-6 levels than the moderate ($p = 0.015$) or high activity subgroups ($p = 0.004$). Similarly, the low activity subgroup also showed lower IL-17 levels compared to the moderate ($p = 0.016$) or high activity ($p = 0.0003$) subgroups.

Leptin levels positively correlated with DAS28 (rho = 0.513, $p < 0.0001$, Table 2) but not with age, the time since onset of disease or BMI. There was also an important correlation between Leptin and IL-1β (rho = 0.585, $p < 0.0001$), suggesting a relationship between this adipocytokine and activation of the immune response. Significant correlations were also observed with Resistin (rho = 0.274, $p < 0.05$) and TNFα (rho = −0.277, $p < 0.005$). Likewise, Resistin levels showed a positive relationship with DAS28 (rho = 0.403, $p < 0.0001$) but not with the other clinical parameters. Additionally, Resistin only showed a positive significant association with Leptin (rho = 0.277, $p < 0.001$) and IL-6 levels (rho = 0.256, $p < 0.05$). For its part, the same analysis confirmed the absence of an association of Adiponectin with disease activity (rho = −0.151, $p > 0.05$, Table 2), however it showed a negative correlation with TNFα (rho = −0.418) and IL-17 (−0.283, $p < 0.05$), and a positive correlation with IL-1β (rho = 0.596).

Both IL-6 and IL-17 showed a significant positive correlation with DAS28 (rho = 0.313, $p < 0.005$; rho = 0.373, $p < 0.0001$ for IL-6 and IL-17, respectively). On the other hand, TNFα levels were not associated with clinical activity (Table 2). For its part, a significant correlation between DAS28 and IL-1β (rho = 0.213, $p < 0.05$) was seen, although it was not possible to differentiate by activity subgroups (Table 1).

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