Value of Serum Macrophage Migration Inhibitory Factor (Mif), Adiponectin, and Other Adipokines as Markers of Proteinuria and Renal Dysfunction in Lupus Nephritis: A Cross-sectional Study.

Jorge Ivan Gamez-Nava  
Universidad de Guadalajara, Centro Universitario de Ciencias de la Salud, Programa de Doctorado en Farmacología y Programa de Doctorado en Salud Publica

Valeria Diaz-Rizo  
Instituto Universitario de Ciencias de la Salud, Departamento de Disciplinas Fisiológicas, Metodológicas e Instrumentales

Edsaul Emilio Perez-Guerrero  
Universidad de Guadalajara, Centro Universitario de Ciencias de la Salud, Instituto de Investigación en Ciencias Biomédicas

Jose Francisco Muñoz-Valle  
Universidad de Guadalajara, Centro Universitario de Ciencias de la Salud, Instituto de Investigación en Ciencias Biomédicas

Ana Miriam Saldaña-Cruz  
Universidad de Guadalajara, Centro Universitario de Ciencias de la Salud, Departamento de Fisiología

Nicte Selene Fajardo-Robledo  
Universidad de Guadalajara, Centro Universitario de Ciencias Exactas e Ingenierías

Heriberto Jacobo-Cuevas  
Universidad de Guadalajara, Centro Universitario de Ciencias de la Salud, Programa de Doctorado en Farmacología

Cesar Arturo Nava-Valdivia  
Universidad de Guadalajara, Centro Universitario de Ciencias de la Salud, Departamento de Microbiología y Patología

Miriam Fabiola Alcaraz-Lopez  
Instituto Mexicano del Seguro Social

Xochitl Trujillo  
Universidad de Colima

Miguel Huerta  
Universidad de Colima

Ernesto German Cardona-Muñoz  
Universidad de Guadalajara, Centro Universitario de Ciencias de la Salud, Departamento
Laura Gonzalez-Lopez (✉ lauraacademicoudg@gmail.com)
a. Universidad de Guadalajara, Centro Universitario de Ciencias de la Salud, Programa de Doctorado en Farmacología y Programa de Doctorado en Salud Pública, Guadalajara, Jalisco, Mexico. Zip Code 44340
https://orcid.org/0000-0002-6517-6344

Research

**Keywords:** Lupus Nephritis, Lupus Erythematosus Systemic, MIF, Adiponectin, Adipokines, Biomarkers

**DOI:** https://doi.org/10.21203/rs.3.rs-51704/v1

**License:** This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Background

To date, there is controverted the association between serum macrophage migration inhibitory factor (MIF) and serum adipokines with Lupus Nephrites.

Objective

To investigate the value of serum MIF, leptin, adiponectin and resistin levels as markers of proteinuria and renal dysfunction in lupus nephritis

Methods

Design: cross-sectional. We included 196 Systemic Lupus Erythematosus (SLE) and 52 controls (HC). In SLE, disease activity was assessed by SLEDAI. Renal involvement was investigated by Renal-SLEDAI: a) Renal-SLE involvement (n=43) and b) Non-Renal-SLE (n=153). MIF, adiponectin, leptin and resistin levels were quantified by ELISA. We assessed correlations of MIF, adipokines and renal involvement parameters [proteinuria (g/day), serum creatinine and other]. Multivariable linear regression was used for investigating factors associated with the intensity of proteinuria.

Results

SLE patients had higher MIF (p=0.02) and adiponectin levels (p<0.001) than HC. In renal-SLE involvement there were higher adiponectin levels (19.0 vs 13.3 µg/mL, p=0.002) and resistin levels (10.7 vs 8.9 ng/mL, p=0.01), compared with non-Renal-SLE. Proteinuria correlated with high adiponectin (r_s=0.19, p<0.009) and resistin levels (r_s=26, p<0.001). MIF (r_s=0.27, p=0.04) and resistin (r_s= 0.18; p=0.02) correlated with increased creatinine. High Renal activity correlated with adiponectin (r_s=0.21, p=0.004). Multiple linear regression identified that elevated adiponectin (p=0.02), younger age (p=0.04) and low MIF (p=0.02) were associated with intensity of proteinuria. Low MIF and high adiponectin levels have interaction for intensity of proteinuria (R^2=0.41).

Conclusions

High adiponectin combined with low MIF concentrations interacts for increase proteinuria in renal-SLE, and MIF and resistin levels are associated with renal dysfunction. These findings highlight the relevance of including the assessment of MIF and adipokines as clinical markers in the evaluation of LN.

Background

Systemic lupus erythematosus (SLE) is considered a chronic inflammatory autoimmune disorder characterised by an extensive spectrum of organ involvement and disease severity. Renal involvement occurs in 30% to 80% of SLE patients [1]. The cumulative incidence of renal involvement in SLE is 54%
[2] Lupus nephritis (LN) is associated with significant morbidity and mortality, with an incidence of end-stage renal disease (ESRD) of 27.6 per 1,000 patient-years [3]. Hispanic patients have a high predisposition to LN similar to Asian and African American populations [4]. Proteinuria is one of the primary clinical markers for LN and is a major prognostic factor of ESRD [5]. Traditional markers of LN, such as increased native double-stranded DNA antibody (anti-dsDNA), and decreased C3 and C4 complement fractions, are currently used in the clinical assessment of LN [6]. However, these markers have not sufficient sensitivity for detecting a relapse of LN in all SLE patients [7]. Non-traditional markers of LN include increased serum levels of some adipokines and cytokines. Some of these markers have been associated with proteinuria, decrease in glomerular filtration rate, impairment of creatinine clearance, haematuria, increase in urinary leucocytes or casts [8–11]. The macrophage migration inhibitory factor (MIF) is an interesting cytokine that modulates inflammatory response regulating T-cell proliferation [12]. However, the relationship between MIF levels and LN is discordant [13,14]. Currently, it has been reported a relation between MIF and adipokines. In non-rheumatic population, MIF levels have been observed increased in obesity [15]. Koska et al. observed that the mRNA expression of the MIF gene in adipocytes is negatively associated with levels of adiponectin [16]. However, currently, there is insufficient information regarding the relation of serum MIF levels and adipokines in LN.

More recently, the associations between some adipokines such as leptin, resistin and adiponectin with LN have been investigated. However, some studies have observed discordant results. Hutcheson et al. identified that adiponectin concentrations decrease when at the time that the disease activity increase; whereas they also an increase in serum creatinine correlating with high levels of adiponectin [9]. Being serum creatinine a marker of renal dysfunction; this finding is relevant for hypothesise the possible role of adiponectin levels as a marker of renal dysfunction. More recently, our group has identified a correlation between high adiponectin levels and severity of proteinuria as well as with high creatinine levels in LN [11].

To date, there are insufficient data regarding the possible interrelation of MIF and adipokines levels as markers of renal activity in SLE patients. Therefore, we decided to assess the value of serum MIF, leptin, adiponectin and resistin levels as markers of proteinuria and renal dysfunction in lupus nephritis.

**Methods**

**Study design**

We conducted a cross-sectional study performed in female patients with SLE. These patients met the following inclusion criteria: a) a diagnosis of SLE as corroborated by a rheumatologist, b) met the 1982 American College of Rheumatology criteria of SLE [17], c) age ≥18 years, d) ethnicity of Mexican-Mestizos [18], and e) disease duration of at least one year since the first symptom. We excluded patients with diagnostic overlap syndrome, pregnancy, active infection, and diseases other than SLE that might produce abnormal proteinuria.
Clinical setting

All patients were selected from the lupus cohort of an outpatient rheumatology clinic of one largest secondary-care centre in Guadalajara, Mexico (Hospital General Regional 110, Instituto Mexicano del Seguro Social [IMSS]).

A healthy control (HC) group of 52 females matched by a range of age and ethnicity were select. These controls were clinically healthy subjects recruited from patients visiting the Department of Preventive Medicine for check-ups at the same hospital. HC were included to compare values of MIF and adipokines with the SLE patients.

Clinical evaluations

Three trained rheumatologists-researchers assessed SLE patients using a structured chart, including disease features, comorbid diseases, and current pharmacological treatment. Assessment of disease damage: this was investigated using the Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) [19]. Assessment of disease activity: this was performed using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) [20]. SLEDAI is an index design to assess disease activity in the preceding ten days; with 24 weighted clinical and laboratory variables corresponding to 9 different organ/systems. SLEDAI score varies from 0 to 105. The renal activity was evaluated with the renal-SLEDAI (rSLEDAI) [20], which represents the sum of the renal items of the SLEDAI. rSLEDAI includes the following items proteinuria, pyuria, erythrocyturia, and urine casts; each one is punctuated with 0 meaning absence or 4 points meaning presence; therefore, the maximum rSLEDAI is 16 [20]. Proteinuria greater than 0.5 grams/day was used as the main criterium of the renal activity or in conjunction with any of the following features: persistent haematuria, leucocytes in urine or urine casts by granulocytes or erythrocytes (excluding other causes). SLE with these features conformed the Renal-SLE group and was compared with the Non-Renal group that consisted of SLE patients without any criteria of rSLEDAI. We included the Mexican version of SLEDAI (MEX-SLEDAI) [21]. MEX-SLEDAI is an adaptation of SLEDAI which do not include laboratory immunological parameters such as complements fraction and anti-dsDNA [21]. MEX-SLEDAI score has a score range of 0 to 32.

MIF and adipokine measurements

Blood samples were extracted from SLE patients and HC at the time of clinical evaluation. Samples were stored at room temperature for 30 minutes after sampling. Then, samples were centrifuged at 1300g for 15 min at 4 °C. Serum samples were stored at -80 °C without freeze-thaw cycles for a maximum of 6 months. All serum samples were coded before the measurements. This strategy was made to blind the researchers who assessed the clinical characteristics to the results of MIF and adipokines minimising the risk of measurement bias. Serum MIF levels were quantified using a commercial ELISA kit (R&D™, Minneapolis, USA). The sensitivity of this assay was 0.068 ng/mL. Measurements of adipokines,
including leptin (sensitivity of 7.8 pg/mL), adiponectin (sensitivity of 0.89 ng/mL), and resistin (sensitivity of 0.055 ng/mL), were performed using commercial ELISA kits (R&D™, Minneapolis, USA). If required, we performed a serum sample dilution in those cases of the values above than the highest point on the standard curve. All ELISA measured were performed according to the manufacture instructions. All samples were run in duplicate to improve assay precision.

**Statistical analysis**

Quantitative variables were described as medians (ranges), and qualitative characteristics were described as frequencies (%). The chi-square test (or Fisher's exact test) was utilised for comparison between proportions. Comparison of quantitative variables between the renal-SLE and non-Renal groups was performed using the Mann-Whitney U test. To compare differences in quantitative variables between three groups (Renal-SLE, non-renal SLE, and HC), we used the Kruskal-Wallis test. In this analysis, \( p \)-values for multiple comparisons were adjusted by Bonferroni correction. To identify the correlations between MIF, adipokines and other clinical variables, Spearman's test was computed. A multiple regression analysis was used (Stepwise method) to find those variables associated with proteinuria (g/day). In this model, those variables with biological plausibility and statistical significance <0.20 in univariate analysis were introduced as covariables.

We further explored the possible interaction effect of MIF and adipokines. For this proposed interaction, we constructed individual regression models. Before the construction of interaction models, we first evaluated the collinearity between MIF and adipokines and their product term. We used the mean centring method for testing interactions as described below [22,23]. Briefly, for each of the tested predictor variables, the mean was subtracted before testing the products to represent their interaction. Then, models of interactions of multiplicative terms were tested for each of the transformed variables (correcting for MIF and adipokines). We used SPSS Statistics for Windows (Version 25.0. Armonk, NY: IBM Corp.) and R version 4.0.0 [24] for performing the statistical analyses. Figures were constructed in R using ggplot2 package [25]. \( p \)-value \( \leq 0.05 \) was considered as statistically significant.

**Results**

**Comparison between Healthy Controls vs SLE patients**

This study included 196 SLE patients and 52 HC. Table 1 shows comparisons of the clinical variables between SLE and HC. All SLE and HC were females and Mexican-Mestizo, SLE patients had a similar median of age compared with HC (45 vs 47 years; \( p=0.87 \)). Body mass index (BMI) was not significantly different between SLE and HC (27.3 vs 27.9, respectively, \( p=0.86 \)).

**Characteristics of the patients with SLE.**

In table 1 also included a description of selected characteristics in the total group of SLE. From 196 SLE patients, 28.1% had positive anti-dsDNA antibodies. The median SLEDAI score was 2 points. Seventy-six
(38.8%) SLE patients had active disease (SLEDAI>4). The r-SLEDAI ranges from 0 to 12 points. Forty-three SLE patients (21.9%) presented renal disease activity. All the SLE patients were receiving glucocorticoids, although only 60 (30.6%) were receiving a dosage >10 mg/day. From the total SLE patients, 74% were receiving immunosuppressive therapy.

Comparison of MIF and adipokines levels between SLE and HC.

Figure 1 presents the comparison of MIF and adipokines levels between SLE and HC. Serum MIF and adiponectin concentrations were higher in the SLE patients compared with HC. In SLE were increased MIF levels [9.1 ng/mL (0.6-43.9) vs. 5.3 ng/mL (0.3-32.7), p=0.02]. Adiponectin concentrations were also higher in SLE compared with the HC [14.5 µg/mL (0.6-45.1) vs 10.2 µg/mL (1.6-24.3), p<0.001]. Resistin levels were lower in SLE than in HC [9.1 ng/mL (2.4-37.1) vs 14.3 ng/mL (1.3-55.9)], p<0.001). No differences were identified in the concentrations of leptin between SLE and HC [18.6 ng/mL (1.6-136.5) vs 18.3 (0.31-87.48), p=0.92].

Comparison of MIF and adipokines levels between HC, Renal-SLE vs Non-Renal-SLE

Figure 2 shows the comparison of MIF and adipokines levels between HC, Renal-SLE vs Non-Renal-SLE. The three groups had differences in adiponectin levels (p<0.001). Post-hoc analysis shows that adiponectin concentrations were more elevated in Renal-SLE compared with HC [19.0 µg/mL (7.3-45.1) vs 10.2 µg/mL (1.6-23.4), p<0.001] and Non-Renal-SLE [19.0 µg/mL (7.3-45.1) vs 13.3 µg/mL (0.6-37.0), p=0.002]. Non-Renal-SLE presented higher levels of adiponectin in comparison with HC [13.3 µg/mL (0.6-37.0) vs 10.2 µg/mL (1.6-23.4), p=0.002]. Resistin concentrations were more elevated in HC than in Non-Renal-SLE [14.3 ng/mL (1.3-55.9) vs 8.9 ng/mL (2.5-37.1), p<0.001] and Renal-SLE [14.3 ng/mL (1.3-55.9) vs 10.7 ng/mL (6.2-26.2) p<0.001]. MIF and leptin levels were not significantly different in the three groups.

Comparison of variables between Renal-SLE vs Non-Renal-SLE

Renal-SLE patients were receiving higher doses of prednisone compared with those patients without renal activity [20 mg/day (2.5-75) vs 7.5 mg/day (2.5-50.0), p<0.001], but the frequency of concurrent use of immunosuppressive drugs was similar in Renal-SLE vs Non-Renal-SLE (p=0.64). Furthermore, Renal-SLE and Non-Renal-SLE patients had similar disease durations[43 years (18-62) vs 46 years (18-73), p=0.13]. Other comparisons of variables between Renal-SLE patients and Non-Renal-SLE are described in table 2.

Correlation between MIF and adipokines with clinical variables.

Table 3 describes the correlations of MIF and adipokines with clinical and laboratory variables. Lower serum MIF levels were correlated with increased age (p=0.003), longer duration of SLE (p=0.004). MIF did not correlate with SLEDAl, rSLEDAl, proteinuria and other features. Serum leptin levels correlated with BMI (p<0.001), proteinuria (p=0.01) and with estimated glomerular filtration rate (eGFR) (p=0.02). High concentrations of adiponectin correlated with proteinuria (p=0.009), rSLEDAl (p=0.004), and high score of Mex-SLEDAl (p=0.03). However, adiponectin levels negatively correlated with eGFR (p=0.05). Additionally,
serum adiponectin levels correlated with glucocorticoid dose (p=0.02) and BMI (p<0.001). A correlation was observed between resistin levels with proteinuria (p<0.001), serum creatinine (p=0.02), SLICC/ACR (p=0.01), and glucocorticoid dose (p=0.03). No correlations were observed between serum leptin or resistin and proteinuria, SLEDAI or rSLEDAI.

**Correlation between in MIF and adipokines with parameters of renal activity in the 43 Renal-SLE patients.**

We investigated the correlation between MIF and adipokines levels with parameters of renal activity in Renal-SLE patients. MIF levels correlated with proteinuria in g/day ($r_s = -0.47; p=0.002$), serum creatinine ($r_s = 0.72$), 24-hour creatinine clearance ($r_s = 0.20$), eGFR ($r_s = 0.45; p=0.003$). In Renal-SLE patients adiponectin levels correlated with serum creatinine ($r_s = 0.357; p=0.02$), however no correlations were observed with other renal inflammatory features. These data are not shown in tables.

**Variables associated with the quantity of proteinuria (g/day): Results of the multiple linear regression analysis**

Table 4 demonstrates the findings of the factors associated with the intensity of proteinuria in gr/day obtained in the multiple linear regression analysis. With the enter method, the variables associated with the intensity of proteinuria (g/day) were: glucocorticoid doses (p<0.001), adiponectin levels (p<0.001), MIF levels (p=0.01) and age (p<0.001). Using the stepwise forward method in multivariable linear regression analysis, the factors associated with the intensity of proteinuria in g/day were higher glucocorticoid doses (p<0.001), higher adiponectin levels (p=0.001), lower MIF levels (p=0.005) and younger age (p=0.011). This model was adjusted by glucocorticoid doses, immunosuppressive therapy, disease duration, age, MIF, adiponectin, leptin, and resistin. The $R^2$ and Adjusted $R^2$ for this model were 0.41 and 0.40, respectively.

We tested for interactions in the multiple regression analysis to assess weighted variables associated with the intensity of proteinuria (data not shown). After testing for interactions, we identified interactions between age and adiponectin levels and the interaction of serum MIF and adiponectin levels with the intensity of proteinuria in SLE patients. In the interaction model, higher adiponectin and higher age increasing proteinuria levels. While lower MIF levels interacted with higher adiponectin levels for an increase in proteinuria. The adjusted $R^2$ for the final interaction model was 0.41 and p<0.001.

**Discussion**

We identified that high adiponectin concentrations combined with low concentrations of MIF are associated with proteinuria in LN and that these associations remain after excluding the effects of other adipokines. We also observed that leptin and resistin correlated with proteinuria. High MIF and resistin levels had a correlation with serum creatinine. However, in the adjusted linear regression analysis, only MIF, adiponectin, age, and GCs doses remained associated with the intensity of proteinuria.
Proteinuria is an important biological marker in LN, and a baseline proteinuria >3.5 g/day is a risk factor of ESRD [5]. Patients with persistent proteinuria develop chronic renal disease with an increase in fibrosis in the renal tubules and renal interstitium [26]. MIF concentrations were related to a decreasing of proteinuria in Renal-SLE, and this relationship remained in the multivariable analysis after adjusting for confounders. Instead, MIF concentrations correlated with an increase of serum creatinine, pointing out that MIF is a marker of renal dysfunction. The information related to the association of MIF levels and LN in the literature has been discordant [13,14,27]. Otukesh et al. identified a higher MIF/creatinine ratio in paediatric patients with LN compared with children without LN [13]. Brown et al. identified an increase in urinary MIF observed only in proliferative nephritis, whereas no association was detected between serum or urinary MIF levels with the quantity of proteinuria [27]. Vincent et al. found no associations between urinary MIF levels and LN, although elevated urinary MIF was observed in SLE with high disease activity [14]. These results suggest that the relation of MIF with the presence of LN is complex and requires an assessment of possible interactions with other molecules, including adipokines that could be associated with LN. MIF is a pleiotropic cytokine inductor of the synthesis of TNF-\(\alpha\) and IL-6; additionally, MIF is a modulator of the inflammatory response regulating T-cell proliferation [12]. In-vivo studies have shown that MIF can annul the anti-inflammatory effect of glucocorticoids [28]. Studies in animal models demonstrate that blocking MIF induces a protective effect for the inflammation in adjuvant-induced arthritis [29]. MIF can antagonise the immunosuppressive effect of glucocorticoids [30]. However, to date; it is not clear if immunosuppressive treatments might modify MIF levels, whereas TNF-\(\alpha\) or IFN-\(\gamma\), might increase the release of MIF [31].

In this study, high adiponectin levels were correlated with an increase in proteinuria. Furthermore, we observed a weak correlation between adiponectin and a decrease of eGFR. Hutchenson et al. reported an association between adiponectin renal dysfunction [9]. Nevertheless, many confounders can influence the results. Therefore, we decided to perform a multivariable analysis, including those confounding variables that might affect the results. After this analysis, adiponectin levels remain as a factor associated with proteinuria. Although we tested in the present study other adipokines, only adiponectin remains associated with the intensity of proteinuria in SLE. These findings might reflect that adiponectin levels constituted a risk factor of proteinuria, nevertheless; these findings can also reflect an increase on adiponectin as results of renal inflammation acting the high serum levels as a potential protective homeostatic mechanism. An experimental study performed in cell cultures suggests that adiponectin can protect the development of chronic renal disease due to a decrease in reactive oxygen species and local renal inflammation and fibrosis [32]. Another experimental study performed in cultured podocytes, it has been identified that the adiponectin regulation of inflammation could be mediated through the stimulation of AMP-activated protein kinase (AMPK) and a decrement of NADPH oxidase [33].

We also observed that leptin and resistin correlated with the severity of proteinuria. Several publications have described increased levels of leptin and resistin in SLE patients [9,34–37]. The association of these adipokines with SLE is complex. Two different meta-analyses had opposite results regarding if leptin levels are different in SLE vs controls [38,39]. Regarding resistin, in a metanalysis Huang et al. did not identify differences in resistin levels, comparing SLE and controls [40]. However, Santos et al. found no
association of leptin or resistin with disease activity in SLE [41]. Hutcheson et al. observed that resistin levels were high in SLE with renal dysfunction, including an increase of serum creatinine [9]. Similarly, to the results observed by Hutcheson et al., we identified that serum resistin levels correlated with an increase in creatinine concentrations.

To date, none of the previously published studies evaluated the possible relation of serum adipokines with MIF levels in LN. Therefore, the present study was the first in to assess a possible association of MIF and adipokines with proteinuria in SLE using a multivariable approach. Our findings support the interaction effect of serum levels of adiponectin and decreased MIF levels on proteinuria in SLE. The interaction that we observed between high adiponectin levels and lower MIF levels in patients with proteinuria secondary to LN might contribute to the identification of a different subgroup of patients with LN with more severe activity. However, it also allows us to formulate the question of whether our findings could reflect an adaptation mechanism involving increased adiponectin levels to limit renal damage in SLE nephritis and could be used to plan other therapeutic strategies.

We have some limitations in this work; first cross-sectional design represents only a snapshot of the complex interrelation of these inflammatory molecules with the LN. Therefore, we ignored whether our findings of an increase in adiponectin together with a decrease in MIF levels might occur before renal relapse; or if contrarily adiponectin increased in an attempt to control the inflammatory process reflected by the low MIF levels. Follow-up studies are required to solve this issue. Another limitation is that the majority of our patients had a long SLE duration, and the characteristics of the cytokine and adipokine profile might vary in patients with early SLE who develop nephritis. Another potential limitation is that practically all of the SLE patients included were receiving treatment with glucocorticoids at the time of the study. We cannot exclude the cumulative effects associated with glucocorticoids on these molecules. MIF is a cytokine that has been observed to decrease the effect of glucocorticoids [42]. Since glucocorticoids increase the serum levels of adipokines, other studies are required to control these confounders. Therefore, our results are derived from those patients with SLE who were previously identified and treated, and a future study assessing LN patients with a recent diagnosis and measurement of the molecules prior to starting immunosuppressive treatment is advised. Finally, a comparison with the serum levels of these analytes with urinary levels should be very relevant to identify other associations. Future studies should take into account the importance of this comparison.

**Conclusions**

In conclusion, with a multivariable approach, higher adiponectin serum levels combined with low concentrations of MIF were found to be associated with proteinuria in LN. After excluding the effect of other adipokines or MIF, this interaction remained. Leptin and resistin levels correlated with proteinuria. This study raises a new hypothesis that these molecules together could play an essential role in LN. However, new longitudinal studies are required.

**List Of Abbreviations**
• American College of Rheumatology (ACR)
• BMI: Body mass index
• double-stranded DNA antibody (anti-dsDNA)
• Estimated glomerular filtration rate (eGFR)
• Healthy control (HC)
• Lupus nephritis (LN)
• macrophage migration inhibitory factor (MIF)
• Mexican systemic lupus erythematosus disease activity index (MEX-SLEDAI)
• renal SLEDAI (rSLEDAI)
• \(r_s\): Spearman Rank Correlation
• Systemic lupus erythematosus (SLE)
• Systemic Lupus Erythematosus Disease Activity Index (SLEDAI)
• Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR)

Declarations

Ethics approval and consent to participate

All patients included in this work signed a written informed consent previous the study entry. This study protocol was approved by the Research and Ethic's board of the hospital. Approval reference: R-2011-1301-92. This study followed the recommendations described by the Declaration of Helsinki.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

All the authors declare that there are no conflicts of interest to disclosure.

Funding

This project was financed with funding from the Fondo de Investigación en Salud del Instituto Mexicano del Seguro Social. Grant number: FIS/IMSS/PROT/G12/1135.

Authors' contribution
Jorge Ivan Gamez-Nava: Conceptualization, Investigation, Resources, Funding acquisition, Supervision, Writing - Original Draft, Writing - Review & Editing

Valeria Diaz-Rizo: Investigation, Conceptualization, Writing - Original Draft, Writing - Review & Editing

Edsaul Emilio Perez-Guerrero EE: Formal analysis, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision,

Jose Francisco Muñoz-Valle: Validation, Resources, Writing - Original Draft, Writing - Review & Editing

Ana Miriam Saldaña-Cruz: Investigation, Writing - Original Draft, Writing - Review & Editing

Nicte Selene Fajardo-Robledo: Investigation, Writing - Original Draft, Writing - Review & Editing

Heriberto Jacobo-Cuevas: Investigation, Writing - Original Draft, Writing - Review & Editing

Cesar Arturo Nava-Valdivia: Investigation, Writing - Original Draft, Writing - Review & Editing

Miriam Fabiola Alcaraz-Lopez: Investigation, Writing - Original Draft, Writing - Review & Editing

Xochitl Trujillo: Validation, Writing - Original Draft, Writing - Review & Editing

Miguel Huerta: Validation, Resources, Writing - Original Draft, Writing - Review & Editing

Ernesto German Cardona-Muñoz: Conceptualization, Investigation, Resources, Writing - Original Draft, Writing - Review & Editing, Supervision

Laura Gonzalez-Lopez: Conceptualization, Methodology, Investigation, Resources, Writing - Original Draft, Writing - Review & Editing, Supervision, Project administration

Authors' information

Dr Laura Gonzalez-Lopez and Dr Jorge I. Gamez-Nava, Current Affiliations: Programa de Doctorado en Farmacología del Departamento de Fisiología y Programa de Doctorado en Salud del Publica Depatamento de Salud Pública, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara.

Both authors have currently the status of jubilated of the Instituto Mexicano del Seguro Social (IMSS)

References

1. Borchers AT, Leibushor N, Naguwa SM, Cheema GS, Shoenfeld Y, Gershwin ME. Lupus nephritis: A critical review. Autoimmun Rev 2012;12:174-94.

2. Bastian HM, Roseman JM, Mcgwin G, Alarcón GS, Friedman AW, Fessler BJ, et al. Systemic lupus erythematosus in three ethnic groups. XII. Risk factors for lupus nephritis after diagnosis. Lupus 2002;11:152-60.
3. Plantinga L, Lim SS, Patzer R, McClellan W, Kramer M, Klein M, et al. Incidence of End-Stage Renal Disease Among Newly Diagnosed Systemic Lupus Erythematosus Patients: The Georgia Lupus Registry: ESRD Incidence in SLE Patients. Arthritis Care Res 2016;68:357-65.

4. Hanly JG, O’Keeffe AG, Su L, Urowitz MB, Romero-Diaz J, Gordon C, et al. The frequency and outcome of lupus nephritis: Results from an international inception cohort study. Rheumatology Oxford 2016;55:252-62.

5. Kono M, Yasuda S, Kato M, Kanetsuka Y, Kurita T, Fujieda Y, et al. Long-term outcome in Japanese patients with lupus nephritis. Lupus 2014;23:1124-32.

6. Misra R, Gupta R. Biomarkers in lupus nephritis. Int J Rheum Dis 2015;18:219-32.

7. Soliman S, Mohan C. Lupus nephritis biomarkers. Clin Immunol 2017;185:10-20.

8. Adhya Z, Borozdenkova S, Karim MY. The role of cytokines as biomarkers in systemic lupus erythematosus and lupus nephritis. Nephrol Dial Transplant 2011;26:3273-80.

9. Hutcheson J, Ye Y, Han J, Arriens C, Saxena R, Li Q-Z, et al. Resistin as a potential marker of renal disease in lupus nephritis: An adipokine marker in lupus nephritis. Clin Exp Immunol 2015;179:435-43.

10. Chen D-Y, Chen Y-M, Wen M-C, Hsieh T-Y, Hung W-T, Lan J-L. The potential role of Th17 cells and Th17-related cytokines in the pathogenesis of lupus nephritis. Lupus 2012;21:1385-96.

11. Diaz-Rizo V, Bonilla-Lara D, Gonzalez-Lopez L, Sanchez-Mosco D, Fajardo-Robledo NS, Perez-Guerrero EE, et al. Serum levels of adiponectin and leptin as biomarkers of proteinuria in lupus nephritis 2017;12:e0184056. doi:10.1371/journal.pone.0184056

12. Lang T, Foote A, Lee JPW, Morand EF, Harris J. MIF: Implications in the Pathoetiology of Systemic Lupus Erythematosus. Front Immunol 2015;6:577.

13. Otukesh H, Chalian M, Hoseini R, Chalian H, Hooman N, Bedayat A, et al. Urine macrophage migration inhibitory factor in pediatric systemic lupus erythematosus. Clin Rheumatol 2007;26:2105-7.

14. Vincent FB, Slavin L, Hoi AY, Kitching AR, Mackay F, Harris J, et al. Analysis of urinary macrophage migration inhibitory factor in systemic lupus erythematosus. Lupus Sci Med 2018;5:e000277. doi:10.1136/lupus-2018-000277

15. Dandona P, Aljada A, Ghanim H, Mohanty P, Tripathy C, Hofmeyer D, et al. Increased Plasma Concentration of Macrophage Migration Inhibitory Factor (MIF) and MIF mRNA in Mononuclear Cells in the Obese and the Suppressive Action of Metformin. J Clin Endocrinol Metab 2004;89:5043-7.

16. Koska J, Stefan N, Dubois S, Trinidad C, Considine RV, Funahashi T, et al. mRNA concentrations of MIF in subcutaneous abdominal adipose cells are associated with adipocyte size and insulin action. Int J
17. Tan EM, Cohen AS, Fries JF, Masi AT, Mcshane DJ, Rothfield NF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271-7.

18. Sánchez-Serrano C. Mestizaje e historia de la población en México. Madrid, España: Real Academia de Ciencias Exactas Físicas y Naturales; 1996. p. 173–193

19. Gladman D, Ginzler E, Goldsmith C, Fortin P, Liang M, Sanchez-Guerrero J, et al. The development and initial validation of the systemic lupus international collaborating clinics/American college of rheumatology damage index for systemic lupus erythematosus. Arthritis Rheum 1996;39:363-9.

20. Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH, Austin A, et al. Derivation of the sledai. A disease activity index for lupus patients. Arthritis Rheum 1992;35:630-40.

21. Guzmán J, Cardiel MH, Arce-Salinas A, Sánchez-Guerrero J, Alarcón-Segovia D. Measurement of disease activity in systemic lupus erythematosus. Prospective validation of 3 clinical indices. J Rheumatol 1992;19:1551-8.

22. McClelland GH, Irwin JR, Disatnik D, Sivan L. Multicollinearity is a red herring in the search for moderator variables: A guide to interpreting moderated multiple regression models and a critique of Iacobucci, Schneider, Popovich, and Bakamitsos (2016). Behav Res Methods 2017;49:394-402.

23. Eberly LE. Multiple Linear Regression. In: Ambrosius WT editors. Topics in Biostatistics. Methods in Molecular Biology. Humana Press; 2007. p 165-187

24. R Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2019. Available from: https://www.R-project.org/

25. Wickham H. ggplot2: Elegant Graphics for Data Analysis (Use R!). Springer International Publishing; 2016.

26. Jorge Medina-Rosas, Zahi Touma. Proteinuria: Assessment and Utility in Lupus Nephritis. Orthop Res Physiother 2016;2:1-8.

27. Brown FG, Nikolic-Paterson DJ, Hill PA, Isbel NM, Dowling J, Metz CM, et al. Urine Macrophage Migration Inhibitory Factor Reflects the Severity of Renal Injury in Human Glomerulonephritis. J Am Soc Nephrol. 2002;13 Suppl 1:S7-S13.

28. Calandra T, Bernhagen J, Metz CN, Spiegel LA, Bacher M, Donnelly T, et al. MIF as a glucocorticoid-induced modulator of cytokine production. Nature 1995;377:68-71.

29. Leech M, Metz C, Bucala R, Morand EF. Regulation of macrophage migration inhibitory factor by endogenous glucocorticoids in rat adjuvant-induced arthritis. Arthritis Rheum 2000;43:827-33.
30. Kang I, Bucala R. The immunobiology of MIF: function, genetics and prospects for precision medicine. Nat Rev Rheumatol 2019;15:427-37.

31. Calandra T, Bernhagen J, Mitchell RA, Bucala R. The macrophage is an important and previously unrecognised source of macrophage migration inhibitory factor. J Exp Med 1994;17:1895-902.

32. Ouedraogo R, Wu X, Xu S-Q, Fuchsel L, Motoshima H, Mahadev K, et al. Adiponectin Suppression of High-Glucose-Induced Reactive Oxygen Species in Vascular Endothelial Cells: Evidence for Involvement of a cAMP Signaling Pathway. Diabetes 2006;55:1840-6.

33. Declèves A-E, Mathew AV, Cunard R, Sharma K. AMPK mediates the initiation of kidney disease induced by a high-fat diet. J Am Soc Nephrol JASN 2011;22:1846-55.

34. Garcia-Gonzalez A, Gonzalez-Lopez L, Valera-Gonzalez IC, Cardona-Muñoz EG, Salazar-Paramo M, González-Ortiz M, et al. Serum leptin levels in women with systemic lupus erythematosus. Rheumatol Int 2002;22:138-41.

35. Chen H, Shi B, Feng X, Kong W, Chen W, Geng L, et al. Leptin and Neutrophil-Activating Peptide 2 Promote Mesenchymal Stem Cell Senescence Through Activation of the Phosphatidylinositol 3-Kinase/Akt Pathway in Patients With Systemic Lupus Erythematosus. Arthritis Rheumatol 2015;67:2383-93.

36. Elshishtawy H, Ibrahim SE, Helmi A, Farouk N, Elshinnawy MA. Resistin in systemic lupus erythematosus: Relation to lupus nephritis and premature atherosclerosis. Egypt Rheumatol 2012;34:137-46.

37. Baker JF, Morales M, Qatanani M, Cucchiara A, Nackos E, Lazar MA, et al. Resistin levels in lupus and associations with disease-specific measures, insulin resistance, and coronary calcification. J Rheumatol 2011;38:2369-75.

38. Lee YH, Song GG. Association between circulating leptin levels and systemic lupus erythematosus: an updated meta-analysis. Lupus 2018;27:428-35.

39. Li H, Zhang T, Leng R, Li X, Li X, Pan H. Plasma/Serum Leptin Levels in Patients with Systemic Lupus Erythematosus: A Meta-analysis. Arch Med Res 2015;46:551-6.

40. Huang Q, Tao S-S, Zhang Y-J, Zhang C, Li L-J, Zhao W, et al. Serum resistin levels in patients with rheumatoid arthritis and systemic lupus erythematosus: a meta-analysis. Clin Rheumatol 2015;34:1713-20.

41. Santos FMM, Telles RW, Lanna CCD, Teixeira AL, Miranda AS, Rocha NP, et al. Adipokines, tumor necrosis factor and its receptors in female patients with systemic lupus erythematosus. Lupus 2017;26:10-6.
42. Roger T, Chanson A-L, Knaup-Reymond M, Calandra T. Macrophage migration inhibitory factor promotes innate immune responses by suppressing glucocorticoid-induced expression of mitogen-activated protein kinase phosphatase-1. Eur J Immunol 2005;35:3405-13.

Tables

Table 1. Comparison of clinical variables between SLE patients and healthy controls
| Variable                        | SLE n= 196 | Healthy Controls n=52 | p |
|--------------------------------|------------|------------------------|---|
| Age (years) a                  | 45 (18-73) | 47 (22-54)             | 0.87 |
| Gender b                       | 196 (100)  | 52 (100)               | —  |
| Mexican-Mestizo b              | 196 (100)  | 52 (100)               | —  |
| BMI (kg/m^2) a                 | 27.3 (17.7-40.0) | 27.9 (18.4-47.3) | 0.86 |
| SLE duration (years) a         | 8.3 (2-28) | —                      | —  |
| C3 fraction complement (mg/dL) a | 142.0 (42.0-252.0) | —                     | —  |
| C4 fraction complement (mg/dL) a | 31 (6.6-71.7) | —                      | —  |
| Positive anti-dsDNA b          | 55 (28.1)  | —                      | —  |
| SLEDAI (score) a               | 2 (0-12)   | —                      | —  |
| rSLEDAI (score) a              | 0 (0-12)   | —                      | —  |
| - Renal-SLE patients b         | 43 (21.9)  | —                      | —  |
| SLICC/ACR (score) a            | 1 (0-5)    | —                      | —  |
| MEX-SLEDAI (score) a           | 1 (0-10)   | —                      | —  |
| Glucocorticoids b              | 196 (100)  | —                      | —  |
| - Prednisone >10 mg/day         | 60 (30.6)  | —                      | —  |
| Immunosuppressive drugs b      | 145 (74.0) | —                      | —  |
| - Azathioprine users b         | 91 (46.4)  | —                      | —  |
| - Cyclophosphamide users b     | 13 (6.6)   | —                      | —  |
| - Mycophenolate users b        | 56 (28.5)  | —                      | —  |
| Other drugs (Methotrexate) b   | 27 (13.8)  | —                      | —  |

a Data expressed as medians and ranges (minimum and maximum value). b Data provided in frequencies (percentages). SLE: Systemic Lupus Erythematosus. SLEDAI: original SLE Disease Activity Index, high score indicates higher disease activity. SLICC/ACR Systemic Lupus International Collaborating Clinics/American College of Rheumatology. rSLEDAI: Renal-SLEDAI score (includes proteinuria greater than 0.5 grams in 24 hours, persistent hematuria, leucocytes on urine or urine casts -granulocytes or
erythrocytes), higher score indicates high renal disease activity. MEX-SLEDAI: Version of SLEDAI validated in Mexico. Comparisons between proportions: Chi-square (or Fisher exact test if required). Comparisons between quantitative variables: Mann-Whitney U test.

Table 2. Comparison of clinical variables between SLE patients without proteinuria and SLE patients with proteinuria

| Variables                   | Non-Renal-SLE n=153 | Renal-SLE n=43 | p     |
|-----------------------------|----------------------|----------------|-------|
| Age (years) ^a              | 46 (18-73)           | 43 (18-62)     | 0.13  |
| Disease duration, (years) ^a| 9 (2-28)             | 6 (2-28)       | 0.10  |
| C3 fraction complement (mg/dL) ^a | 142.0 (60.0-142.0)  | 154.5 (42.0-252.0) | 0.84  |
| C4 fraction complement (mg/dL) ^a | 31 (7.4-71.7)      | 31.3 (6.7-62.9) | 0.92  |
| Positive anti-dsDNA ^b      | 39 (25.5)            | 16 (37.2)      | 0.27  |
| SLEDAI (score) ^a           | 2 (0-12)             | 6 (4-12)       | <0.001|
| Creatinine clearance (mL/min) | 123.1 (96.6-158.3)   | 114.9 (80.8-147.0) | 0.06  |
| eGFR (mL/min/m2)            | 111.2 (29.6-258.9)   | 108.6 (17.1-182.9) | 0.91  |
| Serum creatinine (mg)       | 0.7 (0.6-2.2)        | 0.7 (0.4-3.7)  | 0.44  |
| Immunosuppressive drugs ^b  | 112 (73.2)           | 33 (76.7)      | 0.64  |
| Glucocorticoid user ^b      | 153 (100)            | 43 (100)       | –     |
| Glucocorticoid doses (mg/day) ^a | 7.5 (2.5-50.0)  | 20 (2.5-75.0)  | <0.001|
| Immunosuppressive drugs ^b  | 112 (73.2)           | 33 (76.7)      | 0.69  |
| - Azathioprine users ^b     | 73 (47.7)            | 18 (41.8)      | 0.88  |
| - Cyclophosphamide users ^b | 9 (5.9)              | 3 (9.3)        | 0.29  |
| - Mycophenolate users ^b    | 37 (24.2)            | 19 (44.2)      | 0.002 |
| Others drugs (Methotrexate) ^b | 23 (15.0)           | 4 (9.3)       | 0.44º |

^a Data expressed as median and range (minimum and maximum value). ^b Data provided in percentages (n/total patients evaluated). SLE: Systemic Lupus Erythematosus. SLEDAI: SLE Disease Activity Index.
rSLEDAI: Renal SLEDAI. MEX-SLEDAI: Mexican version of SLEDAI. Estimated glomerular filtration rate (eGFR). Renal-SLE includes patients with proteinuria greater than 0.5 grams in 24 hours, as sole criterion or in conjunction with persistent hematuria, leucocytes on urine or urine casts by granulocytes or erythrocytes. Comparisons between proportions were compared with Chi-square or Fisher exact test (when required).

Table 3. Correlations between cytokines and adipokines with clinical variables: including disease activity index, rSLEDAI score, individual markers of renal activity or renal dysfunction and glucocorticoids doses in SLE-patients.

|                         | MIF (ng/mL) n=196 | Leptin (ng/mL) n=196 | Adiponectin (µg/mL) n=196 | Resistin (ng/mL) n=188 |
|-------------------------|-------------------|----------------------|---------------------------|------------------------|
|                         | rs                | p                    | rs                        | p                      |
| Age, years              | -0.21             | 0.003                | 0.04                      | 0.62                   |
| BMI, kg/m²              | -0.06             | 0.36                 | 0.47                      | <0.001                 |
| Disease duration, years | -0.21             | 0.004                | 0.06                      | 0.38                   |
| SLEDAI, score           | 0.04              | 0.54                 | 0.09                      | 0.22                   |
| rSLEDAI, score          | -0.003            | 0.96                 | 0.1                       | 0.17                   |
| MEX-SLEDAI, score       | 0.02              | 0.75                 | 0.1                       | 0.13                   |
| SLICC/ACR, score        | 0.15              | 0.15                 | 0.09                      | 0.24                   |
| Proteinuria, g/24 h     | -0.02             | 0.77                 | 0.18                      | 0.01                   |
| Creatinine, mg          | 0.27              | 0.04                 | 0.12                      | 0.1                   |
| eGFR (mL/min/m2)        | 0.11              | 0.13                 | 0.17                      | 0.02                   |
| Creatinine clearance (mL/min) | 0.07           | 0.39                 | 0.1                       | 0.89                   |
| GCs doses, mg/day       | -0.04             | 0.63                 | 0.1                       | 0.15                   |

rs: Spearman Rank Correlation. BMI: Body mass index. SLE: Systemic Lupus Erythematosus. SLEDAI: SLE Disease Activity Index. rSLEDAI: Renal SLEDAI. MEX-SLEDAI: Mexican version of SLEDAI. SLICC/ACR: Systemic Lupus International Collaborating Clinics/American College of Rheumatology. eGFR: Estimated glomerular filtration rate. Glucocorticoid doses included: prednisone doses or deflazacort doses expressed as equivalent prednisone doses. Spearman Rank Correlation test p < 0.05.
Table 4. Variables associated with intensity of proteinuria in the linear regression analysis

| Independent Variables | Univariable analysis | Multivariable analysis |
|------------------------|----------------------|------------------------|
|                        |                      |                        |
|                        | Proteinuria g/day    |                        |
|                        | Univariable analysis | Multivariable analysis |
|                        |                      |                        |
|                        | β coefficient (IC 95%) | β coefficient (IC 95%) |
| GCs (mg/day)           | 0.07 (0.05 to 0.08)  | <0.001                 |
|                        |                      | 0.05 (0.04 to 0.08)    | <0.001 |
| Adiponectin levels (µg/mL) | 0.09 (0.06 to 0.11) | <0.001                 |
|                        |                      | 0.05 (0.02 to 0.07)    | 0.001  |
| MIF (ng/mL)            | -0.04 (-0.08 to -0.01) | 0.02                  |
|                        |                      | -0.04 (-0.07 to -0.01) | 0.005  |
| Age (years)            | -0.05 (-0.07 to -0.03) | <0.001             |
|                        |                      | -0.03 (-0.05 to -0.01) | 0.011  |
| Leptin, ng/mL          | -0.00 (-0.01 to 0.01) | 0.70                   |
|                        |                      | Not significant to the model |
| Resistin, ng/mL        | 0.03 (-0.02 to 0.09)  | 0.30                   |
|                        |                      | Not significant to the model |
| Immunosuppressive drugs| ---                  | ---                    |
|                        |                      | Not significant to the model |

**Dependent variable:** quantity total of 24-hr proteinuria. Multiple regression analysis was performed using stepwise method. Model was adjusted by disease duration, age, adiponectin, MIF, leptin, resistin, Glucocorticoid doses expressed as equivalent of prednisone doses (GCs) and using of immunosuppressive therapy. $R^2$ for multivariable model was 0.41. Adjusted $R^2$ for multivariable model was 0.40. Covariates included in this analysis were those variables with statistical significance in the univariate analysis or were considered with biological plausibility to proteinuria.

**Figures**
Figure 1

Comparison of MIF and adipokines between healthy controls (HC) and systemic lupus erythematosus (SLE) patients. Comparisons were performed between with Mann-Whitney U test.
Figure 2

Comparison of MIF and adipokines in HC, Renal-SLE, and Non-Renal-SLE. HC: healthy controls. SLE: Systemic Lupus Erythematosus. Renal-SLE includes patients with proteinuria higher than 0.5 grams/day, as sole criterion or in conjunction with persistent hematuria, leucocytes on urine or urine casts by granulocytes or erythrocytes. Comparisons between quantitative variables were performed with the Kruskal-Wallis test. p<0.05. P values for multiple comparisons were adjusted by Bonferroni correction. ns: p > 0.05. *: p <= 0.05. **: p <= 0.01. ***: p <= 0.001. ****: p <= 0.0001