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

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Research

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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 fordetecting 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 SLEpatients 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 ºC 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 usingcommercial 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 describedas 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]. A*p-value* $\leq$0.05 was considered as statistically significant.

Results

Comparison between Healthy Controls vs SLE patients

This study included 196 SLEpatients 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 47years; 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 tree 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 SLEDAI, rSLEDAI, 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), rSLEDAI (p=0.004), and high score of Mex-SLEDAI (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= -0.06; p=0.72), 24-hour creatinine clearance (r_s= 0.21; p=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-α 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, whereasTNF- or IFN-γ, 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.

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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	
	r_s	p	r_s	p	r_s	p	r_s	p
Age, years	**-0.21**	**0.003**	0.04	0.62	-0.13	0.07	0.03	0.67
BMI, kg/m^2	-0.06	0.36	**0.47**	**<0.001**	**-0.27**	**<0.001**	**0.21**	**0.004**
Disease duration, years	**-0.21**	**0.004**	0.06	0.38	-0.07	0.35	0.06	0.42
SLEDAI, score	0.04	0.54	0.09	0.22	0.11	0.13	0.03	0.70
rSLEDAI, score	-0.003	0.96	0.1	0.17	**0.21**	**0.004**	0.13	0.07
MEX-SLEDAI, score	0.02	0.75	0.1	0.13	**0.16**	**0.03**	0.04	0.58
SLICC/ACR, score	0.15	0.15	0.09	0.24	0.03	0.65	**0.19**	**0.01**
Proteinuria, g/24 h	-0.02	0.77	**0.18**	**0.01**	**0.19**	**0.009**	**0.26**	**<0.001**
Creatinine, mg	**0.27**	**0.04**	0.12	0.1	0.16	0.22	**0.18**	**0.02**
eGFR (mL/min/m2)	0.11	0.13	**0.17**	**0.02**	**-0.15**	**0.05**	0.01	0.91
Creatinine clearance (mL/min)	0.07	0.39	0.1	0.89	-0.01	0.18	-0.06	0.44
GCs doses, mg/day	-0.04	0.63	0.1	0.15	**0.17**	**0.02**	**0.16**	**0.03**

r_s: 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

		Proteinuria g/day		
	Univariable analysis		Multivariable analysis	
Independent Variables	β coefficient (IC 95%)	P	β coefficient (IC 95%)	p
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

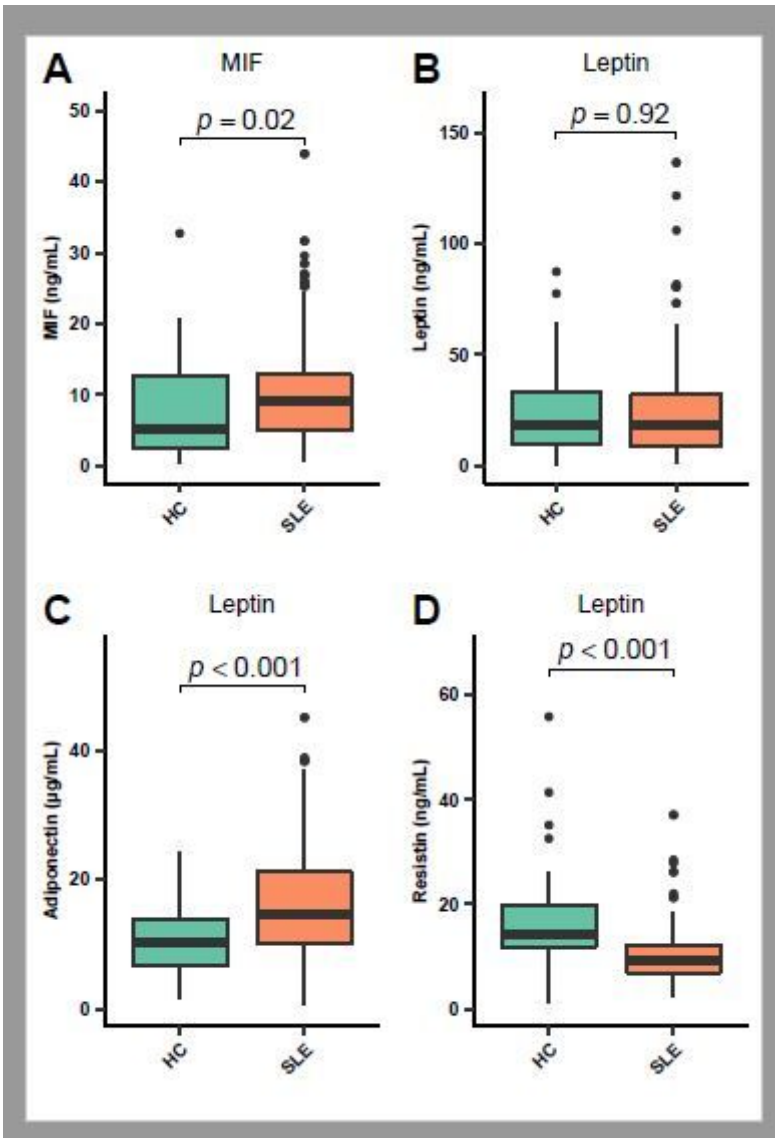

Figure 1

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

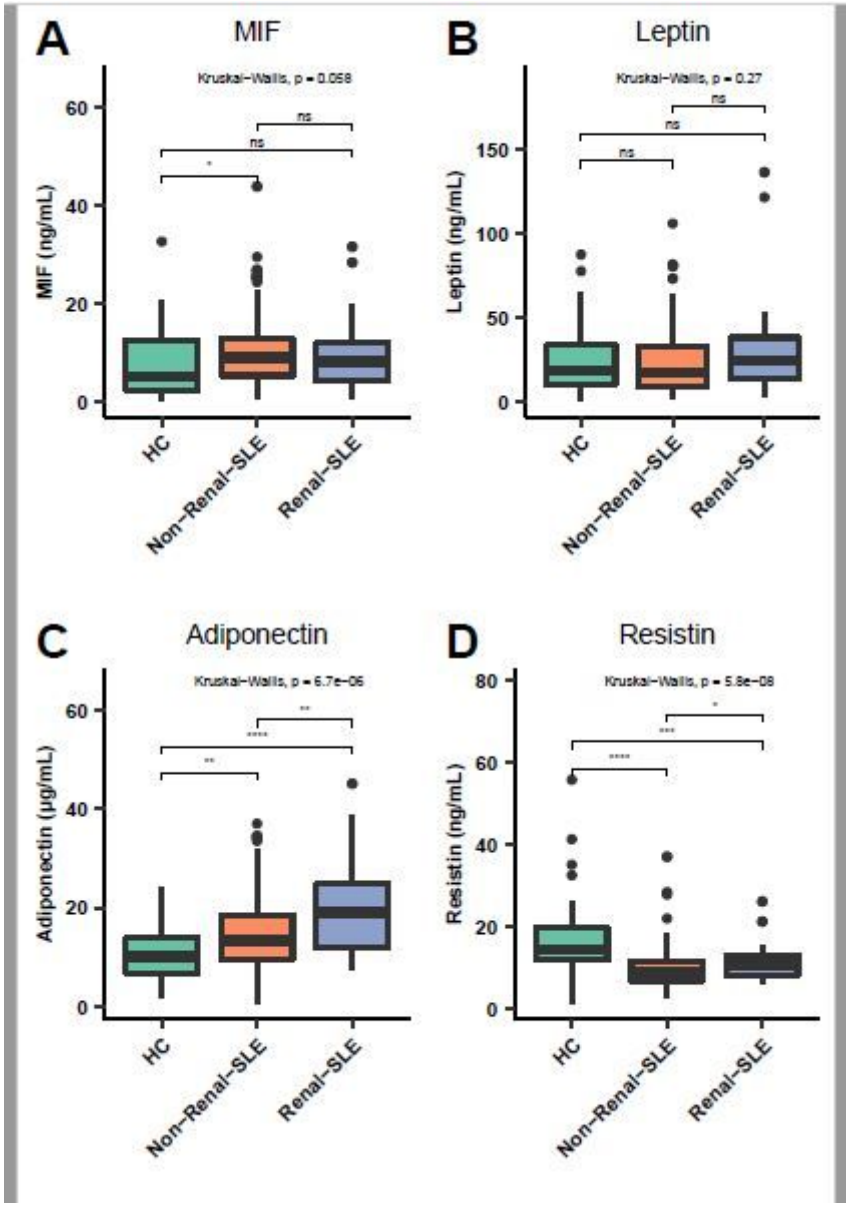

Figure 2

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