



Brief original

MCP-1 in urine as biomarker of renal lupus in absence of cytokines, interferon- γ and growth factors

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ABSTRACT

Objective: To characterize 17 immunological markers in the Urine of patients with SLE.

Introduction: Lupus nephritis is an inflammatory disease affecting the renal parenchyma. Cytokines and chemokines are key immune mediators that have been related with the pathogenesis of the disease. Obtaining non invasive prognosis markers is a highly desirable objective in order to improve the clinical management of these patients.

Patients and methods: In this study we profiled 17 immune mediators (Th1, Th2, Th17 cytokines, chemokines and growth factors) in the urine of 25 patients with systemic lupus erythematosus with active renal disease by using a Biorad[®] 17-plex kit on a Luminex[®] platform. A group of healthy volunteers of similar age and comparable sex distribution was recruited as control (n=10).

Results: Results evidenced that the only detectable mediators in urine were IL-8, MCP-1 and MIP-1 β . When levels of these mediators were compared between patients and controls, significantly higher levels of MCP-1 were observed in the urine of the patients. MCP-1 levels in urine correlated positively with the SLEDAI score in a significant way and negatively with plasma levels of complement C4.

Conclusions: Our results reinforce the role of MCP-1 in urine as biomarker of disease activity in renal lupus, excluding the detection of other soluble immune mediators such as Th1, Th2, Th17 cytokines and growth factors as suitable markers in this non invasive sample.

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MCP-1 en orina como biomarcador de lupus renal en ausencia de citoquinas, interferón- γ y factores de crecimiento

RESUMEN

Objetivo: Perfilar 17 medidores inmunológicos en la orina de pacientes con LES.

Introducción: La nefritis lúpica es una enfermedad inflamatoria que afecta al parénquima renal. Citoquinas y quimiocinas son los mediadores inmunes dominantes relacionados con la patogénesis de la enfermedad. La obtención de marcadores de pronóstico no invasivos es un objetivo sumamente deseable para mejorar el manejo clínico de estos pacientes.

Pacientes y métodos: En este estudio nosotros perfilamos 17 mediadores inmunológicos (citoquinas Th1, Th2, Th17, quimiocinas y factores de crecimiento) en la orina de 25 pacientes con lupus y enfermedad renal activa usando un kit Biorad[®] 17-plex en plataforma Luminex[®]. Como grupo control se seleccionaron (n = 10) voluntarios sanos con similar edad y sexo que los casos.

Resultados: Los resultados evidenciaron que los únicos mediadores perceptibles en orina eran IL-8, MCP-1 y MIP-1 β . Cuando los niveles de estos mediadores fueron comparados entre los pacientes y los controles, MCP-1 en la orina de los casos fue el único que aumentó de forma significativa ($p < 0,05$) con respecto a los controles. Estos niveles de MCP-1 en orina se correlacionaron de forma positiva con la puntuación de SLEDAI y de forma negativa con los niveles en plasma de la proteína C4 del sistema de complemento.

Conclusiones: Nuestros resultados refuerzan el papel de MCP-1 en orina como biomarcador no invasivo de actividad de enfermedad en el lupus renal, no evidenciando la detección de otros mediadores inmunológicos solubles, como son las citoquinas Th1, Th2, Th17 y factores de crecimiento.

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Introduction

Lupus nephritis is an inflammatory disease affecting the renal parenchyma. Cytokine and chemokines are the key immune mediators that have been related with the pathogenesis of the disease.¹ Obtaining non-invasive prognostic markers is a key objective to improve the clinical management of these patients.² The presence of chemokines in the urine of patients with lupus nephritis has been proved.^{3,4} The multiplex assays available today allow for the simultaneous detection of a great variety of mediators in a very small amount of urine. Monocyte chemoattractant protein-1 (MCP-1) is considered a renal inflammation marker, but it is not exclusive to lupus. It is present in high levels in the urine of patients with diabetes mellitus, IgA nephritis, and certain types of vasculitis, among other conditions.

Material and methods

In this study, we profiled 17 molecules present in urine, from the first morning urination, of 25 patients with systemic lupus erythematosus (SLE)¹² with active renal disease by using a Biorad© 17-plex kit on a Luminex® platform. The selected mediators included Th1 cytokines (IL-2; IL-12p70); Th2 cytokines (IL-4; IL-5; IL-10; IL-13); Th17 cytokines (IL-6; IL-17); growth factors (GM-CSF; GCSF); chemokines (IL-8; MCP-1; MIP-1 β), and also IL-7. Patients with SLE were selected from the Autoimmune Disease Unit at our hospital. A group of 10 healthy people who work at the University of Valladolid (Spain) of similar age and comparable gender distribution to that of the group of patients was selected as control group. The definition of patients with SLE was that of the American College of Rheumatology.⁵ All patients with SLE were receiving immunomodulator treatment at the time of sample collection, either with non-steroidal anti-inflammatory drugs, steroids, chloroquine, or immunosuppressive medication. Disease activity in each patient was assessed at the moment of sample collection using the SLE Disease Activity Index 2000.⁶ Renal disease was defined as the presence of proteinuria (>0.5 g/24 h), which is the predominate characteristic in lupus nephritis, of pyuria (>6 leukocytes/field), the presence of urinary casts detected by microscopic examination of urinary sediment, haematuria (>5 red blood cells/field), creatinine levels in blood serum above 1.1 mg/dL for women and 1.3 mg/dL for men, and the clearance of creatinine below 87 mL/min in women or 97 mL/min in men. The best method to evaluate renal function is estimating the glomerular filtration rate (GFR) using the Cockcroft-Gault or MDRD formulae. The results of the estimated GFR are expressed as follows: values higher than 60 mL/min/1.73 m² were reported as GFR higher than 60 mL/min; in the case of GFR equal to or below 60 mL/min/1.73 m², the numerical value calculated by the equation must be noted (Consensus Document of the Spanish Nephrology Society and the Spanish Clinical Biochemistry Society).

The urine sample was collected in an appropriate, sterile Eppendorf tube and was immediately frozen at -80 °C until the immune mediators were evaluated. The mediators were analysed in the infection and immune research unit at our hospital. The Mann-Whitney U test was used to assess the significance of the different levels of mediators between groups. The associations between mediator levels, laboratory parameters, and severity levels were studied by calculating the Spearman-Kärber correlation coefficients. The study was approved by the Review Board of the University Hospital of Valladolid, Spain. Informed consent was obtained from all the study subjects.

Results

The results showed that the only detectable mediators in urine were IL-8, MCP-1, and MIP-1 β . When levels of these mediators were compared between patients and controls, significantly higher levels of MCP-1 were observed in the urine of patients than in controls

(results expressed as median [interquartile range]): patients (124.7 [138.6] pg/mL) and controls (56.8 [75.7] pg/mL) ($P < .05$). No difference was found between patients and controls for IL-8 and MIP-1 β . The positive correlation between the MCP-1 levels in urine and the score of the SLE Disease Activity Index 2000¹³ with statistical significance (expressed as Spearman's correlation coefficient; P) (0.569; 0.004) and the negative correlation with plasma levels of C4 protein of the complement system (-0.389; 0.05) were an interesting discovery. Just as interesting is the discovery of a positive association between the urine levels of MCP-1 and IL-8 (0.436; 0.029) and between MCP-1 and MIP-1 β (0.478; 0.016), which somehow suggests coordinated secretion of these chemokines in this disease.

No relation was found between MCP-1 and the GFR ratio with the MDRD formula of patients, with the mean being 89.57 mL/min/1.73 m².

We simultaneously examined 17 immune mediators in the urine of patients with renal disease in the context of SLE for the first time.

Discussion

Chemokines, such as MCP-1 and IL-8, are powerful chemotactic factors of monocytes in the glomerulus when there is renal damage.⁸⁻¹¹ Furthermore, MCP-1 stimulates the increase of intracellular calcium, which induces the freeing of superoxide anions and lysosomal enzymes and stimulates the production of adherence molecules and cytokines. Many cytokines and pro-inflammatory mediators could cause the production of MCP-1 in the mesangial cells of the glomerulus, the endothelial cells of tubules, and the monocytes. It has been proven that increases of tubular MCP-1 expression were strongly associated to infiltration of monocytes and fibrosis in the interstice of patients with lupus nephritis. This suggests that MCP-1 takes part in the pathogenesis of tubular-interstitial damage, as it recruits monocytes and the fibrosis of the interstice takes place.

One method to detect the expression of MCP-1 in glomerular disease is to measure the levels of MCP-1 in urine, which is high in patients with glomerulopathies, and more acutely so in patients who present inflammatory glomerulopathies. Our results reinforce the role of MCP-1 in urine as a biomarker of disease activity in lupus nephritis; the production of MCP-1 is minimal or undetectable in the renal tissue of subjects without renal affection, excluding the detection of other soluble immune mediators such as Th1, Th2, and Th17 cytokines and growth factors as non-invasive markers.

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Conflict of interest

The authors declare no conflict of interests.

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