Original Research Article

The Egyptian Journal of Immunology

The Egyptian Journal of Immunology
Volume 31 (1), 2024: 116–123.
www.Ejimmunology.org

The role of urinary biomarker monocyte chemoattractant protein (MCP-1) in correlation with different histopathological classes of lupus nephritis in Egyptian patients

Howayda ÈI-Shinnawy, Osama Mahmoud, Waleed Abdelmohsen, Amr Ahmed, and Lamis Khedr

Department of Internal Medicine, Nephrology Unit, Faculty of Medicine, Ain Shams University, Cairo 11566, Egypt.

Corresponding author: Amr Ahmed, Department of Internal Medicine, Nephrology Unit, Faculty of Medicine, Ain Shams University, Cairo 11566, Egypt.
Email: amrhikal@med.asu.edu.eg

Abstract

Lupus nephritis (LN) affects almost two-thirds of systemic lupus erythematosus (SLE) patients. Renal biopsy is the gold standard for the diagnosis of LN. However, repeated biopsies are not always performed in clinical practice, and they carry some risk. Therefore, minimally invasive techniques, as urinary biomarkers, are promising tools for the diagnosis and monitoring of SLE. Previous studies evaluated urinary monocyte chemoattractant protein-1 (MCP-1) in patients with SLE, reported higher levels of urinary MCP-1 in patients with active LN than non-active LN. Other studies reported higher levels of urinary MCP-1 in LN patients with proliferative forms (III and IV). This study aimed to evaluate urinary MCP-1 as a noninvasive diagnostic biomarker tool for LN, and to determine its association with different LN histopathological stages and chronicity indices. The study included 40 SLE patients with biopsy-proven LN class II, III, IV or V, and 20 patients with inactive LN as a control group. In LN active patients, the mean creatinine was 1.71 ± 0.55 mg/dl, and 0.84 ± 0.10 mg/dl in the control group. The mean MCP-1 level was 618.4 ± 294.2 ng/l in active LN patients and 120.05 ± 87.53 ng/l in inactive LN patients. The receiver operating characteristic (ROC) curve analysis indicated a better diagnostic performance of MCP-1 than conventional biomarkers. At area under the curve of 0.990, the best cut-off level was >245 ng/L (sensitivity 97.5 %, Specificity 95 %). In conclusion, urinary MCP-1 distinguished active LN from inactive renal disease. It can be proposed as a good noninvasive diagnostic biomarker with a high sensitivity and specificity for detection of LN activity..

Keywords: Urinary monocyte chemoattractant protein (MCP-1), SLE, LN.

Date received: 25 February 2023; **accepted:** 11 December 2023

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease. It is heterogeneous, of unknown etiology and characterized by the production of autoantibodies and the involvement of multiple organ systems. Renal involvement occurs in up to 60% of patients and is a major predictor of morbidity and mortality.[1] Despite advances in the treatment of lupus nephritis (LN), its management is fraught with

uncertainty and lack of reliable biomarkers for intra-renal activity and chronicity.[2]

The pathogenesis of renal microvascular lesions in LN is not fully understood. Results from animal models and human lupus experiments have suggested that the activation and dysfunction of endothelial cells, in addition to the contribution of immune system dysfunction, especially the immune complex-induced vascular inflammation and antiphospholipid antibody-associated thrombotic events, are key mechanisms in the development of renal microvascular lesions in LN that need to be further investigated.[3]

Renal biopsy is the gold standard for the diagnosis of LN. However, repeated biopsies are not always performed in clinical practice, and they carry some risk. For that reason, minimally invasive techniques, such as urinary biomarkers, are a promising tool for the diagnosis and monitoring of SLE.[4]

Consequently, many serum and urinary biomarkers have been studied in SLE patients for the complementary study of LN. Thus, conventional biomarkers for the determination of LN like proteinuria, protein/creatinine ratio in spot urine, 24 h urine proteinuria, creatinine clearance, double-chain anti-DNA levels, and serum complement, among others have been extensively researched in LN.[5] Nevertheless, these biomarkers may not be useful enough to appropriately predict relapse, accurately monitor response to treatment, or identify the degree of disease activity and chronic damage. For this reason, investigations for new potential biomarkers that overcome these obstacles are clearly necessary.[6]

Monocyte chemoattractant protein-1 (MCP-1) is composed of 76 amino acids and belongs to a family possessed of at least four members (MCP-1, MCP-2, MCP-3, and MCP-4). MCP-1 is produced by renal mesangial cells, endothelial cells, tubular epithelial cells, and smooth muscle cells in response to several cytokines. MCP-1 is a potent chemoattractant for monocytes, activated CD4 and CD8 memory T lymphocytes. In addition, MCP-1 induces granule release from natural killer cells and CD8 T cells. In murine models of LN, genetic depletion or blockade of MCP-1 ameliorates glomerular and interstitial inflammation and hence renal damage.[7]

Markowitz et al., 2007, evaluated urinary MCP-1 in patients with SLE, reported significantly higher levels of urinary MCP-1 in patients with active LN than in non-active LN. In addition, some authors reported higher levels of urinary MCP-1 in patients with proliferative forms (III and IV) of LN.[8]

Liu L et al., 2020, showed that urinary measurement of MCP-1 correlated more precisely with the renal domain-score of the SLE disease activity index (rSLEDAI), being even more accurate than the measurement of C3 and C4 in serum.[9] For the current study, we hypothesized that urinary MCP-1 would significantly correlate with different histopathological stages of LN.

This study was designed to determine whether urinary MCP-1 could be a cheap and noninvasive diagnostic tool for patients with active LN. Consequently, we assessed the relation between MCP-1 urinary biomarker and different histopathological stages of LN, disease activity and chronicity indices.

Patients and Methods

Study population

Our study included 60 SLE patients who were admitted in the Rheumatology and Nephrology Units at Ain shams University Hospitals. They included 40 SLE patients with active LN, and 20 lupus patients with inactive LN as a control group. The patients fulfilled at least four of the EULAR/ACR 1982 revised criteria to diagnose SLE.[10] All patients were randomly selected from Rheumatology and Nephrology Units at Ain shams University Hospitals during 2021 and 2022. We excluded diabetics, patients with non-lupus related renal affection, patients on hemodialysis, renal transplant patients, drug-induced lupus, overlapping syndromes, urinary tract infection, active systemic infection, and active malignancies.

The 60 study patients were divided into two groups based on LN activity. The first group included 40 SLE patients with renal involvement: proteinuria of > 0.5 gram/day, active urinary sediments (cellular elements,

namely red or white blood cells, or the same cells in "cast" form), estimated glomerular filtration rate (eGFR) ≥30 ml/min/1.73m². The second, control group included 20 patients with inactive lupus, complete responders i.e., normal or ≤25% decline of modification of diet in renal disease (MDRD)-GFR from baseline and a proteinuria of less than 0.5 g/day.

Clinical and laboratory measurement

All participants were subjected to detailed history taking, including sociodemographic data (age, sex, and residence). All the patients were diagnosed according to the American College of Rheumatology (ACR) diagnostic criteria. The disease activity was assessed by the Systemic Lupus Erythematosus Disease Activity Index renal (SLEDAI-R) score (minimum score is 0 and the maximum is 16).[11] The scores are corresponding to the presence of any one of the following concerning urine analyses: hematuria, proteinuria, pyuria, or urinary red cell casts.

Chronicity index scores were calculated from the summation of individual scores. The range of chronicity index score was 0 to 12 with higher scores representing higher chronicity [12]. Data for complete blood cell count, serum creatinine level, blood urea nitrogen (BUN) were obtained from patient's hospital records.

Assessment of urine MCP-1 by an enzyme-linked immunosorbent assay (ELISA) technique

The first morning voided, midstream urine samples were collected in a sterile container. The sample was centrifuged to remove sediments then kept frozen in aliquots at −80℃ for later urinary MCP-1 testing. Commercially available ELISA kits (No.6497, Sun Red Biotechnology Laboratory, hutia Road, Baoshan District, Shanghai, China) were used for assessment of urine MCP-1 according to the manufacturer's instructions. The optical density (OD value) of the human monocyte chemoattractant protein-1/monocyte chemotactic and activating factor (MCP-1/MCAF) ELISA Kit final products were determined using a microplate reader (model 0125 201, by Sun Red Biotechnology Laboratory in China) set to 450 nm. The OD values of the samples were plotted on the standard curve and MCP-1 antibody titers were obtained.

Statistical Analysis

The collected data were coded, tabulated, and statistically analyzed using the Statistical Package for Social Sciences (SPSS) software (version 28.0), (IBM Corp., Chicago, USA, 2021). Quantitative data were tested for normality using the Shapiro-Wilk test, then described as mean ±SD (standard deviation) as well as minimum and maximum of the range. They were compared using independent t-test (two independent groups) and ANOVA test (three independent groups). Qualitative data are described as number and percentage and compared using the Chi square test as well as Fisher's Exact test for variables with small, expected numbers. The receiver operating characteristic (ROC) curve was used to evaluate the performance of different tests to differentiate between certain groups. The Bonferroni test was used for post hoc comparisons. The level of significance was considered at p-value < 0.05 as significant.

Results

Our study included 60 SLE patients, 40 SLE patients with renal involvement with mean age 28.75 ± 6.37 years. Female patients were 33 (82.5%) and 7 (17.5%) male patients. The duration of SLE ranged from recently diagnosed patients up to 8 years and the mean was 37 ±29.5 months. The control group included 20 patients with inactive LN Table 1.

Table 1. Comparison of demographic data between the two studied groups.

	Active LN (n= 40)		Inactive LN (n= 20)		*p* value
	No.	%	No.	%	
Sex					
Male	7	17.5	0	0.0	^{FE}NS
Female	33	82.5	20	100.0	
Age (years)					
Min. – Max.	19.0 – 44.0		18.0 – 42.0		
Mean ± SD.	28.75 ± 6.37		27.70 ± 6.44		NS
Median (IQR)	29.0 (22.50 - 33.50)		27.50 (23.0 - 32.0)		
Body weight (kg)					
Min. – Max.	46.0 – 78.0		47.0 – 78.0		
Mean ± SD.	63.70 ± 8.05		62.90 ± 8.05		NS
Median (IQR)	64.0 (58.0 – 69.50)		63.50 (58.0 – 67.50)		

P > 0.05 is not significant (NS).

Basic demographic, clinical and laboratory data between the two study groups

There was no difference in age, sex, weight, and duration of SLE disease between the two study groups. However, disease clinical manifestations including lower limb edema and hematuria were significantly higher in the active LN patients than controls. There was no difference between the two groups of patients as regard the class of LN and in activity index in renal biopsy, however chronicity index was significantly higher in active LN patients.

As regards blood picture data, hemoglobin was statistically significantly higher in patients without LN. Albumin was significantly lower in active LN group than the inactive LN group. Serum BUN and creatinine levels were significantly higher in the active LN group than the inactive LN group (p<0.001). eGFR was significantly lower in the active LN group (Table 2).

Table 2. Comparison of laboratory data between the two study groups.

Studied parameter	Active LN (n= 40)	Inactive LN (n= 20)	*p* value
BUN (Mean ± SD)	50.93 ± 24.78	24.50 ± 9.56	<0.001
Creatinine (Mean ± SD)	1.71 ± 0.55	0.84 ± 0.10	<0.001
Albumin (Mean ± SD)	2.89 ± 0.61	4.05 ± 0.42	<0.001
eGFR (Mean ± SD)	56.14 ± 20.40	99.63 ± 6.90	<0.001
Hemoglobin (gm/dL) (Mean ± SD)	9.42 ± 1.84	10.17 ± 0.99	0.044
TLC (x10^3/mL) (Mean ± SD)	6.99 ± 3.75	6.45 ± 1.95	NS
Platelets (x10^3/mL) (Mean ± SD)	225.1 ± 88.81	260.5 ± 74.46	NS

P > 0.05 is not significant (NS).

Urine MCP-1 and urinary findings of the study patients

In the active LN group, 18 patients (45 %) had active urinary sediment and 22 patients (55 %) had no active urinary sediment. All 20 patients in the control group had inactive urinary sediment. When comparing kidney function tests there were significant statistical differences between the two study groups. The mean BUN was 50.93 ± 24.78 mg/dl in the active cases, and 24.50 ± 9.56 mg/dl in the control group. The mean creatinine was 1.71 ± 0.55 mg/dl in the active cases, and 0.84 ± 0.10 mg/dl in the control group. The mean albumin was 2.89 ± 0.61 mg/dl in the active cases, and 4.05 ± 0.42 mg/dl in the control group. The mean eGFR was 56.14 ± 20.40 ml/min/1.73 m in

the active cases, and 99.63 ± 6.90 40 ml/min/1.73 m in the control group.

Our study showed a significant correlation between disease activity and urinary MCP-1 level (Table 3). The mean MCP-1 level was 618.4 ± 294.2 ng/l in patients with active LN and 120.05 ± 87.53 ng/l in patients with inactive LN (Figure 1) with a sensitivity of 97.5% and specificity 95 % (Table 4).

Table 3. Comparison of MCP-1 Level between the two studied groups.

MCP-1 Level	Active (n= 40)	Inactive (n= 20)	*p* value
Mean ± SD	618.4 ± 294.2	120.05 ± 87.53	<0.001

*$P \leq 0.05$ is significant.

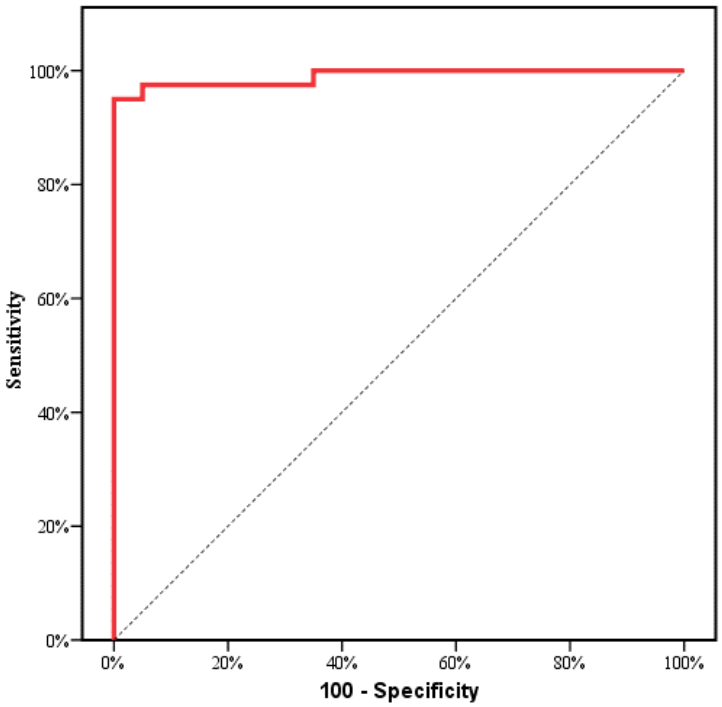

Figure 1. Receiver operating characteristic (ROC) curve analysis for MCP-1 Level to detect active lupus nephritis.

Table 4. Prognostic performance for MCP-1 Level to detect active lupus nephritis.

	AUC	*p* value	95% C. I	Cut off	Sensitivity	Specificity	PPV	NPV
MCP-1 Level	0.990	<0.001	0.971 – 1.0	>245	97.50	95.0	97.5	95.0

PPV: positive predictive value; NPV: negative predictive value. $P \leq 0.05$ is significant.

Our study also revealed that there was a statistically significant correlation between level of MCP-1 and histopathological class of lupus nephritis. Level of urinary MCP-1 was highest in class IV with mean ± SD of 988.0 ± 177.55, median of 998.5 followed by class III with mean ± SD of 592.07 ± 112.05, median of 567 then class V with mean ± SD of 351.60 ± 32.66, median of 357.5 and the least was class II with

mean ± SD of 268.75 ± 40.9, median of 270 (Table 5 and Figure 2).

Table 5. Relation between MCP-1 Level and class of lupus nephritis in the 40 active patients.

	No	MCP-1 Level			*p* value
		Min. – Max.	Mean ± SD.	Median	
Class of lupus nephritis					
II	4	222.0 – 313.0	268.75 ± 40.94	270.0	<0.001
III	14	429.0 – 810.0	592.07 ± 112.05	567.0	
IV	12	717.0 – 1280.0	988.0 ± 177.55	998.50	
V	10	302.0 – 390.0	351.60 ± 32.66	357.50	
Active urinary sediment					
No	22	222.0 – 1095.0	487.41 ± 250.65	380.0	0.001
Yes	18	302.0 – 1280.0	778.50 ± 267.48	735.0	

*$P \leq 0.05$ is significant.

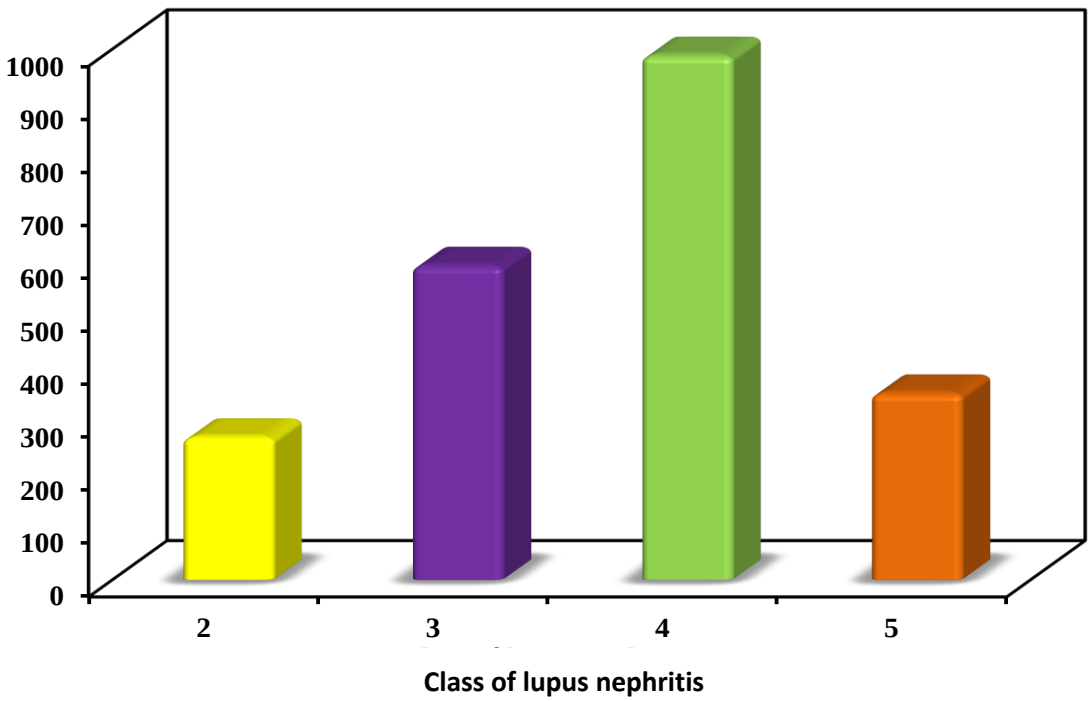

Figure 2. Relation between urine MCP-1 Level and class of lupus nephritis in the active group.

Discussion

This study was designed to determine whether urinary MCP-1 could be a noninvasive diagnostic tool for patients with active LN. Consequently, we assessed the relation between MCP-1 urinary biomarker and different histopathological stages of LN, disease activity and chronicity indices.

Aragón et al., 2020, reviewed LN potential renal biomarkers and found that MCP-1 urinary biomarker was associated with disease activity and evaluated with tools, such as rSLEDAI,

suggesting that high levels of MCP-1 indicate active LN.[13]

In our study, we chose using urinary MCP-1 with sensitivity of 97% and a specificity of 100% in detecting active lupus nephritis. However, a study by Taha et al., 2017, reported that glomerular MCP1 had a sensitivity of 64% and specificity of 95%.[14] We found statistically significant correlation between level of MCP-1 and SLEDAI-Renal score with (p=0.002). The findings of a study by Abujam et al., 2013, agreed with ours that MCP-1 positively correlated with rSLEDAI (p<0.001).[15] In addition, a study by Gómez-Puerta et al., 2018, reported that levels of MCP-1 were significantly elevated in renal active (rSLEDAI ≥4) patients rather than renal inactive (rSLEDAI < 4) patients (p < 0.01).[16] Another study showed that urinary measurement of MCP-1, correlated more precisely with the rSLEDAI, being even more accurate than measurement of serum C3 and C4.[17] This may suggest that urinary MCP-1 could be added to the panel of non-invasive biomarkers used for detecting renal flares.[14] Our study also revealed that there is a statistically significant correlation between the level of MCP-1 and the histopathological class of LN. This was consistent with findings of a study by Dong et al., 2018, which revealed that the levels of uMCP-1 were varied in patients with different biopsy classification.[17]

Finally, our study revealed that the levels of uMCP-1 were 111.12 ± 58.92 pg/mg Cr in class II nephritis patients, 224.86 ± 168.70 in class III (including III+V) patients, 229.70 ± 130.04 in class IV (including IV+V) patients, 308.07 ± 248.98 in class V (including V+III and V+IV) patients. In conclusion, urinary MCP-1 was able to distinguish active from inactive renal disease. It has a consistently good diagnostic performance with a high sensitivity and specificity for detection of LN activity. Urinary MCP-1 also provides an auxiliary noninvasive tool to distinguish between different histopathological classes of active LN. Thus, it could be added to the panel of biomarkers

Acknowledgements

The authors would like to express their thanks to the staff working in the Outpatient Clinic and Inpatient Ward Departments and all the patients and their families who helped us to perform this study..

Author Contributions

The study investigators were HE, OM, WA, LK and AA, all proposed the topic of this research and designed the study. AA collected the data. All the authors contributed to preparation of the final draft of the manuscript, revised the manuscript and critically reviewed the intellectual contents. In addition, they have all read and approved the final manuscript and are responsible for its accuracy and integrity.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) denies receipt of any financial support for the research, authorship, and/or publication of this article.

Ethical approval

The study protocol was reviewed and approved by the Research Ethics Committee of Faculty of Medicine of Ain Shams University (FMASU MD 281/2020).

Informed consent

A whiten informed consent was obtained from each study patient before participating in the study.

ORCID iD

Amr Ahmed iD https://orcid.org/0000-0003-4492-0389.

References

1. AA Gasparin, N Pamplona Bueno de Andrade, V Hax, et al. (2019). Urinary biomarkers for lupus nephritis. *Lupus* 28, 265–272.
2. Lan L, Han F, Lang X, Chen J. (2016). Monocyte chemotactic protein 1, fractalkine, and receptor for advanced glycation end products in different pathological types of lupus nephritis and their value in different treatment prognoses. *PLoS One*; 11: e0159964a.

3. Atehortua L, Rojas M, Vasquez GM, et al. (2017). Endothelial alterations in systemic lupus erythematosus and rheumatoid arthritis: potential effect of monocyte interaction. *Mediators Inflamm*. 9680729.
4. Wu H, Zeng J, Yin J, et al. (2017). Organ-specific biomarkers in lupus. Autoimmun Rev 16: 391–397.
5. Aragón C. C., Tafúr R. A., Suárez-Avellaneda A., et al. (2020). Urinary biomarkers in lupus nephritis. *Journal of translational autoimmunity* 3, 100042.
6. JA Go´ mez-Puerta, B Ortiz-Reyes, T Urrego, et al. (2018). Urinary neutrophil gelatinase-associated lipocalin and monocyte chemoattractant protein 1 as biomarkers for lupus nephritis in Colombian SLE patients. *Lupus* 27, 637–646.
7. Liu L, Wang R, Ding H, et al. (2020). The utility of urinary biomarker panel in predicting renal pathology and treatment response in Chinese lupus nephritis patients. *PLoSONE* 15(10): e0240942.
8. Markowitz GS, D'Agati VD. (2007). The ISN/RPS 2003 classification of lupus nephritis: An assessment at 3 years. *Kidney Int* 71:491-5.
9. Bombardier C., Gladman D.D., Urowitz M.B., et al. (2006). Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. *Arthritis Rheum*, 35: 630- 40.
10. Aringer, Martin. (2019). EULAR/ACR classification criteria for SLE. Seminars in *Arthritis & Rheumatism* 49(3): 14-17.
11. Gladman DD, Ibañez D, Urowitz MB. (2002). Systemic lupus erythematosus disease activity index 2000. *J Rheumatol.* 29(2):288_291.
12. Austin HA 3rd, Muenz LR, Joyce KM, et al. (1984). Diffuse proliferative lupus nephritis: identification of specific pathologic features affecting renal outcome. *Kidney Int.* 25(4):689_695. doi:10.1038/ki.1984.75.
13. Aragón, C. C., Tafúr, R. A., Suárez-Avellaneda, A., et al. (2020). Urinary biomarkers in lupus nephritis. In Journal of Translational Autoimmunity (Vol. 3). *Elsevier* B.V. https://doi.org/10.1016/j.jtauto.2020.100042.
14. Taha, H. A., Abdallah, N. H., Salem, M. N., et al. (2017). Urinary and tissue monocyte chemoattractant protein1 (MCP1) in lupus nephritis patients. *Egyptian Rheumatologist*, 39(3), 145–150. https://doi.org/10.1016/j.ejr.2017.01.004.
15. Abujam, B., Cheekatla, S. S., Aggarwal, A (2013). Urinary CXCL-10/IP-10 and MCP-1 as markers to assess activity of lupus nephritis. *Lupus,* 22(6), 614–623. https://doi.org/10.1177/0961203313484977.
16. Gómez-Puerta, J. A., Ortiz-Reyes, B., Urrego, T.et al. (2018). Urinary neutrophil gelatinase-associated lipocalin and monocyte chemoattractant protein 1 as biomarkers for lupus nephritis in Colombian SLE patients. *Lupus*, 27(4), 637–646. https://doi.org/10.1177/0961203317738226.
17. Dong X, Zheng Z, Luo X, et al. (2018). Combined utilization of untimed single urine of MCP-1 and TWEAK as a potential indicator for proteinuria in lupus nephritis, *Medicine* (Baltim.) 16 1–7.