Serum and Urine Monocyte Chemoattractant Protein-1 as A Markers for Lupus Nephritis

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Lupus nephritis (LN) is a common major organ manifestation and main cause of morbidity and mortality of the disease. We aimed to determine the level of serum and urinary monocyte chemoattractant protein-1(sMCP-1 and uMCP-1) in systemic lupus erythematosus (SLE) patients with and without LN and analyze their association with different clinical and serologic parameters of disease activity. We enrolled 60 female patients with SLE (32 with LN and 28 without LN) and 20 controls.MCP-1 and anti-dsDNA were measured by ELISA. There was statistically significant increase in serum and urinary MCP-1 in all SLE patients (mean=711.59, 676.68 pg/ml respectively) as compared to the control group (mean= 635.70, 632.40 pg/ml respectively), *P*=0.034, 0.020 respectively. Among patients with LN there was statistically significant increase in sMCP-1 (mean=723.58) compared to the control group (*P*=0.038, and in uMCP-1 (mean=699.08) compared to patients without LN (mean=651.07) and control group (mean=632.40), *P*=0.007, 0.002 respectively. Urinary, but not serum MCP-1, positively correlated with 24 hour proteinuria, anti-dsDNA, renal SLEDAI ,biopsy activity index (r=0.362, *P*=0.004; r=0.303, *P*=0.019; r= 0.267, *P*=0.039; r=0.353, *P*=0.047 respectively) and negatively correlated with serum albumin (r=-0.329, *P*=0.010).There was statistically significant increase in uMCP-1 and anti-dsDNA in patients with poor response compared to patients with good response to immunosuppressant therapy (*P*= 0.025; *P*=0.034 respectively). In conclusion, uMCP-1 is associated with LN and disease activity and may be used as a useful tool for diagnosis and follow up.

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by multi-organ damage and the production of autoantibodies directed against multiple cellular components [1-2]. Lupus nephritis (LN) occurs in up to 60% of adults with SLE, and up to 30% of LN patient's progress to end-stage renal disease (ESRD) [3].

LN is a common major organ manifestation and main cause of morbidity and mortality of the disease [4]. Therefore, an involvement of renal disease activity is one of the most important prognostic factors for patients with SLE, and the diagnosis of SLE patients with LN has an important clinical implication in guiding the treatment of SLE in clinical settings [5].

Renal biopsy remains the cornerstone for diagnosis due to the presence of a significant correlation between histological findings, early diagnosis and therapy [6]. In contrast, current noninvasive laboratory markers for LN such as proteinuria, urine protein to creatinine ratio, creatinine clearance, anti-dsDNA and complement levels are unsatisfactory because they lack sensitivity and specificity for differentiating renal activity and damage in LN [7].

The search for an accurate and reliable biomarker for LN is particularly important since the only reliable method to evaluate it is by performing a kidney biopsy, that is an invasive procedure may not always be feasible. So a significant effort has been put into identifying biomarkers that can anticipate impending lupus renal flare,

forecast development of chronic kidney disease, or reflect kidney histology at time of flare [8].

It is thought that urinary biomarkers are superior to the serum ones for LN, probably because they are direct products and the consequence of inflammation or injury to the kidney [9-10].Among them, it seems that monocyte chemoatractant protein-1(MCP-1) has got a prominent place as one of the newest markers of LN activity [11-12]. MCP-1 is a chemokine that attracts monocytes/macrophages to sites of inflammation [13]. MCP-1 is produced by mesangial, podocyte, and monocyte cells in response to various proinflammatory stimuli such as tumor necrosis factor alpha (TNF-α). These inflammatory cells and substances subsequently mediate tissue injury and contribute to the development of renal dysfunction [13].

The aim of this study was to assess serum and urine MCP-1 in SLE with and without LN and evaluate their association with different clinical & laboratory disease activity parameters.

Patients and Methods

The present study included sixty female patients with SLE and twenty age and sex matched healthy volunteers as controls. The patients were included if fulfilling at least four criteria of SLE according to American College of Rheumatology (ACR) [14] and diagnosis of LN was done according to certain parameters such as the presence of persistent proteinuria>500 mg/24 hours, or 3+ count in occasional urine sample [15]. All the patients were females, their ages ranged from 18 to 47 years.

The patients were classified into the following groups:

1- Group1: 32 SLE patients with renal biopsy proven LN.

2- Group2: 28 SLE patients without renal affection.

All patients were selected from outpatient clinic of rheumatology unit and Internal Medicine Department Assiut University Hospitals.

Patients with diabetes mellitus; overlap syndrome (coexistence of lupus with other connective tissue diseases such as rheumatoid arthritis or scleroderma), urinary tract infection, and ESRD were all excluded.

The study received approval from the Ethics Committee of the Faculty of Medicine, Assiut University, and an informed consent was obtained from each subject before enrolment in the study.

Methods

Full history taking stressing on renal symptoms, history of hypertension, duration of disease and type of treatment.

Thorough clinical examination and clinical assessment of the disease activity using SLEDAI-2k [16]. Assessment of Renal SLEDAI: Kidney disease activity is assessed by rSLEDAI score that consists of the 4 kidney-related items of the SLE Disease activity (hematuria, pyuria, proteinuria and urinary casts). The presence of each one gives a score of 4 points; thus, the score can range from 0 (non-active renal disease) to a maximal score of 16 [17].

To all the studied groups the following investigations were performed

Laboratory investigations: 24 hours proteins in urine, Creatinine clearance was evaluated using Cockcroft-Gault Formula, Glomerular filtration was evaluated using the modification of diet in renal disease (MDRD) formula, Complement 3& 4, Antinuclear antibody (ANA)., Anti-double strand DNA (Anti-dsDNA), Estimation of serum and urinary MCP-1 by human MCP-1 ELIZA kit Sinogeneclon CO., Ltd, Catalog No: SG 10069.

Beside Renal biopsy and histopathological examination and classification according to 1995 modified WHO classification [18] to LN patients.

The patients with lupus nephritis were followed up for 6 months after receiving treatment to assess response to immunosuppressant with repeated serum Creatinine, e GFR and 24hours urinary protein after 6 months.

Complete renal response, defined as urine protein: creatinine ratio (UPCR) <50 mg/mmol (roughly equivalent to proteinuria <0.5g/24h) and normal or near normal (within 10% of normal GFR if previously abnormal) GFR. Partial renal response, defined as ≥50% reduction in proteinuria to subnephrotic levels and normal or near-normal GFR,

should be achieved preferably by 6 months and no later than 12 months following treatment initiation [19]. Based on the response of treatment, two subgroups were identified:

-Subgroup1 (n=16) with good response (include those with complete and partial renal response)

-Subgroup2 (n=16) with poor response to standard immunosuppressant therapy (patients who could not achieve complete/ partial remission at the end of 3months with the standard immunosuppressant therapy of prednisone +cyclophosphamide)

Statistical Analysis

Date entry and data analysis were done using SPSS version 22 (Statistical Package for Social Science). Data were presented as number, percentage, mean, standard deviation. Chi-square test and Fisher Exact test were used to compare between qualitative variables. Independent samples t-test was used to compare quantitative variables between two groups and Pearson correlation was done to measure correlation between quantitative variables in case of parametric data. Mann-Whitney test was used to compare quantitative variables between groups, Wilcoxon Signed Rank Test was done to compare quantitative variables between before and after treatment and Spearman correlation was done to measure correlation between quantitative variables in case of non-parametric data. Med calc Program was used to calculate sensitivity, specificity, positive and negative predictive values. P-value considered statistically significant when $P<0.05$.

Results

Renal biopsy was done for 32 patients and we found 4 patients with class II Mesangial proliferative LN, 5 patients with class III Focal proliferative LN, 19 patients class IV Diffuse proliferative LN and 4 patients were class V Membranous LN. The Median Activity Index /24 score was 9.0 and median Chronicity Index /12 score was 2.

All The studied patients were females. After proper examinations, SLE patients were stratified into:

Group1: Included 32 female SLE patients with renal disease based on the results of the renal biopsy. Their mean age was 27.53±7.80 years while; their Median disease duration was 12.0 (2.5-42.0) months. ANAs were positive in 31(96.9%). Mean±SD of anti-dsDNA antibodies was 77.84±48.51. Eleven (34.4%) patients had SLEDAI scores from 0 to 10 while 21(65.6%) had SLEDAI ≥11.

Group2: Included 28 female SLE patients, who had never had clinical and/or laboratory evidence of major renal manifestation attributable to SLE. Their mean age was 28.82±7.91 years while, their Median disease duration was 21.0 (2.0-36.0) months. ANAs were positive in 26(92.9%). Mean±SD of anti-dsDNA antibodies was 50.29±20.27. Twenty two (78.65) patients had SLEDAI scores from 0 to10 while 6(21.4%) had SLEDAI ≥11 (Table 1).

All patients received Hydroxychloroquine and calcium/vitamin D supplementation. In addition, all patients with class 1I Mesangial proliferative LN were treated with steroids alone. For induction all patients with class 3, 4 and 5 received 1gm IV pulse methyl prednisolone/d for 3days and beside this five patients with proliferative lupus nephritis (classes 3 Focal proliferative LN and class 4 Diffuse proliferative LN) received mycophenolate while the other 19 patients received monthly high-dose cyclophosphamide (750 mg/m2/month) for 6 months. All patients with class5 membranous nephropathy were given monthly high dose cyclophosphamide for 6 doses. For maintenance, all the patients who chose mycophenolate for induction also continued it during maintenance phase but other patients with Focal proliferative, Diffuse proliferative, and membranous LN were treated with azathioprine for maintenance.

Table 1. Demographic data of SLE patients and controls.

Variable	SLE with LN (n= 32)	SLE without LN (n= 28)	Controls (n= 20)	*P*-value[1]	*P*-value[2]	*P*-value[3]
Age (years):						
Mean ± SD	27.53 ± 7.80	28.82 ± 7.91	28.50 ± 6.82	NS	NS	NS
Range	18 – 47	18 – 42	18 – 40			
Hypertension: No. (%)	6 (18.8%)	3 (10.7%)	0 (0.0%)	NS	NS	NS
Duration of disease: (months)						
< 12	14 (43.8%)	12 (42.9%)		NS		
12 - < 36	10 (31.3%)	11 (39.3%)				
≥ 36	8 (25.0%)	5 (17.9%)				
Median (IQR)	12.0 (2.5-42.0)	21.0 (2.0-36.0)		NS		

N: number %: percentage SD: standard deviation IQR: inter quartile range

P-value[1]: between patients with LN and without LN *P*-value[2]: between patients with LN and control

P-value[3]: between patients without renal affection and control

P>0.05 is not significant (NS).

SLE disease activity index (SLEDAI) score in the studied group of patients

All patients have different degrees of activity ranging from mild, moderate and high to very high activity according to the score of SLEDAI2k. There was statistically significant increased number of patients with mild activity in patients without LN compared to patients with LN with *P*=0.001 while there was statistically significant increased number of patients with high activity in LN patients compared to patients without LN with *P*=0.009.

As regard proteinuria in the 24 hours urine samples, it was less than 0.5gm in patients without LN, while in LN patients, 24 had levels between 0.5-3gm and 8 patients >3gm. There was statistically significant increase in blood urea in patients with LN compared to those without LN (*P*=0.011), while creatinine clearance was insignificantly increased in patients without LN compared to patients with LN (Table 2).

There was significant increase in mean±SD ESR 1st and 2nd hour in patients with and without LN compared to control group (*P*=0.000), and significant increase in mean Anti-dsDNA in patients with LN compared to patients without LN (*P*=0.007) (Table 3).

Table 2. Urine analysis and kidney function in SLE patients with and without LN

Variable	SLE with LN (n=32)		SLE without LN(n=28)		*P*-value
	N	%	N	%	
Urine analysis:					
Pus cells:					0.007*
≤ 5 / HPF	17	53.1	24	85.7	
> 5 / HPF	15	46.9	4	14.3	
RBCs:					
≤ 5 / HPF	21	65.6	25	89.3	0.031*
> 5 / HPF	11	34.4	3	10.7	
Albumin:					
Negative	0	0.0	22	78.6	0.000*
+	8	25.0	6	21.4	
++	12	37.5	0	0.0	
+++	11	34.4	0	0.0	
++++	1	3.1	0	0.0	
Casts:					
Positive	14	43.8	0	0.0	0.000*
Negative	18	56.2	28	100.0	
Protein in 24 hrs urine:					
< 0.5 gm	0	0.0	28	100.0	0.000*
0.5 – 3 gm	24	75	0	0.0	
> 3 gm	8	25	0	0.0	
Urea: mmol/ L: Median (IQR)	7.0 (4.2-11.0)		5.0 (3.7-6.0)		0.011*
Creatinine (μmol/L): Median (IQR)	66.5 (56.0-123.0)		69.0 (52.5-78.0)		NS
Creatinine clearance (ml/min): Median (IQR)	123.5 (70.0-153.5)		129.0 (99.0-148.5)		NS
eGFR: mL/min: Median (IQR)	116.5 (54.0-143.5)		115.0 (90.5-147.5)		NS

N:number;%:percentage;SD:standard deviation; RBCs:red blood cells; IQR:interquartile range;

eGFR:estimated glomerular filtration rate

P>0.05 is not significant (NS).

Table 3. Laboratory parameters of disease activity parameters in SLE patients and controls.

	Patients		Controls			
	SLE with LN (n= 32)	SLE without LN (n= 28)	(n= 20)	*P*-value[1]	*P*-value[2]	*P*-value[3]
	Mean ± SD	Mean ± SD	Mean ± SD			
ESR 1st	65.94 ± 33.50	64.18 ± 38.29	4.90 ± 1.59	NS	0.000*	0.000*
ESR 2nd	93.72 ± 31.88	84.86 ± 34.21	10.35 ± 1.73	NS	0.000*	0.000*
C3	0.90 ± 0.46	0.97 ± 0.46	--	NS	--	--
C4	0.21 ± 0.19	0.20 ± 0.11	--	NS	--	--
Anti -ds DNA	77.84 ± 48.51	50.29 ± 20.27	--	0.007*	--	--
ANA:	No. %	No. %	No. %			
Positive	31 96.9	26 92.9	0 0.0	NS	0.000*	0.000*
Negative	1 3.1	2 7.1	20 100.0			

N: number; %:percentage; SD:standard deviation; ESR:erythrocyte sedimentation rate; Anti-dsDNA: anti double strand DNA; C3, C4: complement 3,4; ANA: antinuclear antibody; P-value[1]: between patients with LN and without LN; P-value[2]: between patients with LN and control; P-value[3]: between patients without renal affection and control. *P*>0.05 is not significant (NS).

There were statistically significant increased serum and urinary MCP-1 in all SLE patients (mean=711.59, 676.68 respectively) compared to control group (mean=635.70, 632.40 respectively) with p=0.034, 0.020 respectively. There was statistically significant increased sMCP-1 in patients with LN (mean=723.58) compared to control group (*p*=0.038). There was statistically significant increased uMCP-1 in patients with LN (mean=699.08) compared to patients without LN (mean=651.07) and control group (mean=632.40) with *P*=0.007, 0.002 respectively (Table 4).

Table 4. Serum and urine MCP-1 levels among patients and controls.

	sMCP-1 (pg/ml)	uMCP-1 (pg/ml)
	Mean ± SD	Mean ± SD
All SLE patients	711.59 ± 125.29	676.68 ± 69.81
SLE with LN (n= 32)	723.58 ± 131.49	699.08 ± 66.85
SLE without LN (n= 28)	697.89 ± 118.69	651.07 ± 65.14
Controls (n= 20)	635.70 ± 164.60	632.40 ± 78.34
P-value	0.034*	0.020*
P-value [1]	0.433	0.007*
P-value [2]	0.038*	0.002*
P-value [3]	0.135	0.373

P-value: comparison between all SLE patients and control, *P-value*[1]:between patients with LN and without LN, *P-value*[2]:between patients with LN and control,*P-value*[3]: between patients without LN and control. *P*>0.05 is not significant (NS).

Regarding correlations of sMCP-1 with other activity markers no significant correlation were found. A moderate significant positive correlation was found between uMCP-1 and 24 hr proteinuria, anti-dsDNA, renal SLEDAI, biopsy activity index(r=0.362, *P*=0.004; r=0.303, *P*=0.019; r=0.267, *P*=0.039; r=0.353, *P*=0.047 respectively), while moderate significant negative correlation between uMCP-1 and serum albumin was found (r=-0.329, *P*=0.010) (Table 5).

Table 5. Correlations of serum and urine MCP-1 between laboratory investigations in SLE patients.

	sMCP-1 (pg/mL)		uMCP-1 (pg/mL)	
	r-value	*P*-value	r-value	*P*-value
24 hours proteinuria	-0.031	NS	0.362	0.004*
Serum albumin	0.030	NS	-0.329	0.010*
Anti- ds DNA	-0.187	NS	0.303	0.019*
Renal SLEDAI	-0.010	NS	0.267	0.039*
Biopsy activity index	0.129	NS	0.353	0.047*

Anti-dsDNA: anti double strand DNA;SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; MCP-1:monocyte chemoatractant protein-1. *P*>0.05 is not significant (NS).

sMCP-1 at cut-off>590.5 pg/ml showed 93.75% sensitivity and 29.17% specificity for the diagnosis of LN with a diagnostic accuracy of 55%. For uMCP-1, at cut-off>662.5, showed 75% sensitivity and 58.33% specificity for the diagnosis of LN, with a diagnostic accuracy of 65% (Table 6).

Table 6. Specificity and sensitivity of serum and urine MCP-1 for diagnosis of LN.

	sMCP-1	uMCP-1
Cut-off (pg/ml)	> 590.5	> 662.5
Sensitivity	93.75	75.00
Specificity	29.17	58.33
+PV	46.9	54.5
-PV	87.5	77.8
Accuracy	55.00	65.00
AUC	0.592	0.705
95% CI	0.476-0.701	0.693-0.802
P-value	NS	

+PV:positive predictive value; -PV:negative predictive value; AUC:area under curve;95% CI:95% confidence interval

P>0.05 is not significant (NS).

There was statistically significant increase in uMCP-1 in patients with poor response to immunosuppressant therapy compared to patients with good response to immunosuppressant therapy with *P*= 0.025

In LN patients with poor prognosis there were significant increase in Anti-ds DNA and Class IV as compared to LN patients with good prognosis (*P*= 0.034, 0.012 respectively).

In patients with good response to immunosuppressant therapy there was significant decrease in the 24hour urinary proteins after 6months follow up compared to the same patients before treatment (P=0.000), while in patients with poor response to immunosuppressant therapy there was significant decrease in eGFR after 6m follow up as compared to the same patients before treatment (P=0.034).

Discussion

LN occurs in up to two-thirds of patients, with 25–50% of patients presenting with clinical renal disease at the time of diagnosis [20] .Despite advances in its management, it is still a major cause of mortality and morbidity with 10–30% of patients progressing to ESRD [20,21].

The results of this study showed that the mean values of serum and urinary MCP-1 in SLE patients were significantly higher compared to controls (P<0.05) our finding in concordance with Živković *et al.* [22].

In the present study sMCP-1 was significantly increased in all SLE patients compared to control group and also there was statistically significant increased level in patients with LN compared to control group but insignificant increased level was found between patients with LN compared to patients without LN. In consistent with our study Lit *et al.,* [23] found that the sMCP-1 was significantly higher in all SLE patients than controls. Also Živković *et al.,* in [22] showed that the median values of sMCP-1 in patients with and without LN did not show any significant difference.

The present study showed that uMCP-1 in patients with LN was significantly higher than both patients without LN and control, While they were insignificantly increased in patients without LN than the control group (P>0.05). This indicates that the difference between all SLE patients and controls was primarily due to patients who had LN. These results were in agreement with Alzawawy *et al.,* [9]. Also in agreement with Rovin *et al.,* [24] who found that the mean level of uMCP-1 at the time of renal flares was significantly higher than that of controls. Singh *et al.,* [25] reported that uMCP-1 could distinguish those patients with active LN from those with inactive renal disease or stable SLE.

Torabinejad *et al.,* [12] noticed that uMCP-1 values could discriminate between different groups of SLE patients according to whether they had LN or not regardless of their SLE activity. Mirfeizi *et al.,* [26] found that uMCP-1 level was significantly higher in patients with LN than in patients without LN. Lit *et al.,* [23] suggested that urine chemokines could serve as biomarkers for renal disease flare and that the lack of significant increase in the circulating levels of sMCP-1 in patients with nephritis was due to the possibility of excretion of locally produced MCP-1 into urine rather than circulating in the blood, and to the extremely short half-life of sMCP-1.

In this study uMCP-1 levels correlated directly with proteinuria. Our finding is in concordance with Alzawawy *et al.,* [9]. Our findings are also in line with those reported by Živković *et al.,* [22] who found that Urinary, but not sMCP-1, positively correlated with proteinuria (r=0.839; P<0.001). In Contrary to our study Mirfeizi ., [26] was unable to establish a correlation between uMCP-1 and proteinuria.

The current study revealed a highly significant positive correlation of uMCP-1 with rSLEDAI score(r=0.267, P=0.039) and insignificant correlation with the total SLEDAI score. Abujam *et al.,* [27] found that uMCP-1 positively correlated with rSLEDAI (P<0.001). The results of

Živković *et al.,* [22] demonstrated that both serum and urine MCP-1 significantly correlated with global SLE activity, In addition, uMCP-1 had a stronger correlation (P<0.001) than sMCP-1 (P<0.01). Barbado *et al.,* [28] found significant positive correlation of uMCP- l with total SLEDAI score. However Chan *et al.*, [29], Rovin *et al.*, [24], Kiani *et al.*, [30] and Gupta *et al.*, [31] found significant correlations between uMCP-1 with both global SLEDAI-2K and renal SLEDAI-2K scores.

In active LN patients in the present study, a significant correlation was found between uMCP-1 and the biopsy activity index while there was insignificant correlation with the chronicity scores. Our results agree with the results of Brunner *et al.,* [32]. Our findings are also in line with Torabinejad *et al.,* [12] who reported that uMCP-1 correlated positively and significantly with the histological activity index. Similar findings have been noted by Rovin *et al*., [24]. These results further support the notion that uMCP-1 may contribute to the development of renal lesions. However in contrary to our results Chan *et al.*, [29] reported that uMCP-1 did not correlate with the histologic activity index. Also Susianti *et al.,* [33] showed no correlation between uMCP-1 with activity and chronicity index.

In the current study a significant positive correlation was found between uMCP-1 and anti-dsDNA in consistent with the study of Kiani *et al.*, [30] and Jason *et al.*, [34].Contradictory to this a studies done by Watson *et al.,* and Živković *et al.*, [10, 22] found that there were no associations between uMCP-1 levels and anti-dsDNA Ab titres.

In this work the sensitivity and specificity of uMCP-1 for diagnosis of LN were 75% and 58.33% respectively and AUC (95%confidence interval) 0.705(0.693-0.802), While the sensitivity and specificity of sMCP-1 were 93.75% and 29.17% respectively.

The present study showed that there was statistically significant increased basal level of uMCP-1 in patients with poor response to immunosuppressant therapy compared to patients with good response to immunosuppressant therapy after follow up 6 months, and therefore may predict the response to immunosuppressant therapy. In contrary to our results Lan *et al.,* [35] found that the lower urine and serum MCP-1 could predict the poor response to immunosuppressants prior to therapy.

In conclusion, urine MCP-1 is associated with LN and disease activity and may be used as a useful tool for diagnosis and follow up.

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References

1. Liu Y, Cui Y, Zhang X, *et al.* Effects of salvianolate on bone metabolism in glucocorticoid-treated lupus-proneb6. Mrl-fas(lpr)/jmice. Drug Des Develelopment Thearapy. 2016; 10:2535–2546.
2. Emamikia S, Gentline C, Chatzidionysiou K, Arnaud L, van Vollenhoven R. Relationship between glucocorticoid dose and adverse events in systemic lupus erythematosus:data from arandomized clinical trial. Scand J Rheumatol. 2018; 47(2):131–140.
3. Jorge A, Wallace ZS, Zhang Y, *et al.* All-Cause and Cause-Specific Mortality Trends of End-Stage Renal Disease Due to Lupus Nephritis from 1995 to 2014. Hoboken, NJ: Arthritis & Rheumatology; 2018.
4. Yap, D. Y. and Lai, K. N. Pathogenesis of Renal Disease in Systemic Lupus Erythematosus-the Role of Autoantibodies and Lymphocytes Subset Abnormalities. International Journal of Molecular Science. 2015; 16:7917-7931

5. Moroni, G., Quaglini, S., Radice, A., Trezzi, B., Raffiotta, F., Messa, P., Sinico, R.A. The value of a panel of autoantibodies for predicting the activity of lupus nephritis at time of renal biopsy, J. Immunol. Res. 2015 (2015) Article ID 106904, 8 pages, 2015.
6. Farah, R. I. Glomerulonephritis pattern at a jordanian tertiary care center. Inter J Nephrol., vol. 2018, pp.1–7, 2018.
7. Mok CC: Biomarkers for Lupus Nephritis: A Critical Appraisal. J Biomed. & Biotechnol. 2010; 638413.
8. Brad H, Rovin Z, Xiaolan Z. Biomarkers for Lupus Nephritis: The Quest Continues, Clin. J. Am. Soc. Nephrol. 2009; 4:1858-1865.
9. Alzawawy A, Zohary M., Ablordiny M., M. Eldalie. Estimation of monocyte-chemoattractantprotein-1 (Mcp-1) level in patients with lupus nephritis. Inter J. Rheum Dis, 2009; 12(4):311‑ 318.
10. Watson L, Midgley A,Pilkington C *et al*. Urinary monocyte chemoattractant protein1 and alpha1 acid glycoprotein as biomarkers of renal disease activity in juvenile-onset systemic lupus erythematosus. Lupus. 2012; 21(5):496–501.
11. Rahman A. Can measuring urinary biomarkers improve the management of lupus nephritis? Arthritis Research Therapy. 2012; 14(6):127
12. Torabinejad, S., Mardani, R., Habibagahi, Z., Roozbeh, J., Khajedehi, P., Pakfetrat, M., Banihashemi, M. A., Banihashemi, S. J. Urinary monocyte chemotactic protein-1 and transforming growth factor-β in systemic lupus erythematosus. Ind. J. Nephrology. 2012; 22 (1):5–12.
13. Matrat A., Veysseyre-Balter C., Trolliet P. *et al*., "Simultaneous detection of anti-C1q and anti-double stranded DNA autoantibodies in lupus nephritis: predictive value for renal flares," Lupus, 2011; 20(1):28–34.
14. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1997; 40(9):1725.
15. Hahn BH, MC Mahon MA, Wilkonsin A *et al.* American College of Rheumatology guidelines for screening, treatment, and management of lupus nephritis. Arthritis Care & Research. 2012; 64(6):797–808.
16. Gladman DD, Ibanez D, Urowitz MB. Systemic lupus erythematosus disease activity index 2000. J Rheumatol. 2002; 29(2): 288-291.
17. Bombardier C, Gladman DD, Urowitz MB.Derivation of the SLEDAI.A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. Arthritis Rheum. 1992; 35(6), 630-640.
18. Churg J, Bernstein J, Glassock R J. Renal disease: classification and atlas of glomerular disease,2nd Ed. New York, Tokyo.1995; 151-156.
19. Reich HN, Gladman DD, Urowitz MB, *et al*. Persistent proteinuria and dyslipidemia increase the risk of progressive chronic kidney disease in lupus erythematosus. Kidney Int. 2011; 79:914–20
20. Maroz N and Segal MS. Lupus nephritis and end-stage kidney disease.Am J Med Sci.2013; 346(4):319–23.
21. Nowling TK and Gilkeson GS. Mechanisms of tissue injury in lupus nephritis. Arthritis Research Therapy.2011;13:250
22. Živković, V., Cvetković, T., Mitić, B., Stamenković, B., Stojanović, S., Radovanović‐Dinić, B., Jurišić, V. Monocyte chemoattractant protein-1 as a marker of systemic lupus erythematosus: an observational study. Rheumatology International. 2017 https://doi.org/10.1007/s00296-017-3888-x.
23. Lit LC, Wong CK, Tam LS, Li EK, Lam CW. Raised plasma concentration and ex vivo production of inflammatory chemokines in patients with systemic lupus erythematosus. Annual Rheum Diseases. 2006; 65:209.
24. Rovin, BH, Song H, Birmingham DJ *et al*. Urine chemokines as biomarkers of human systemic lupus erythematosus activity. J. Am. Soc. Nephrology. Dec 2005; 16(12):467–473.
25. Singh RG, Usha SS. Rathore SK, Behura, Singh NK. Urinary MCP-1 as diagnostic and prognostic marker in patients with lupus nephritis flare. Lupus. 2012; 21(11):1214–1218.
26. MirfeiziZ., Mahmoudi, M. Naghibi, M. Hatef, M., Sharifipour, F., Jokar, M., Zeraati, A., Shariati Sarabi, Z., Azarian, A. Urine monocyte chemoattractant protein-1(UMCP-1) as a biomarker of renal involvement in systemic lupus

erythematosus, Iran J Basic Med. Sci. 2012; 15(6)1191–1195.

27. Abujam, B., Cheekatla, S., Aggarwal, A. Urinary CXCL-10/IP-10 and MCP-1 as markers to assess activity of lupus nephritis. Lupus. May 2013; 22(6):614–623.

28. Barbado J, Martin D,Vega L, *et al.* MCP-1 in urine as biomarker of disease activity in systemic lupus erythematosus. Cytokine.2012; 60(2):583–586

29. Chan RW, Lai FM, Li EK, Tam LS, Wong TY, Szeto CY, Li PK and Szeto CC. .Expression of chemokine and fibrosing factor messenger RNA in the urinary sediment of patients with lupus nephritis. Arthritis Rheum. Sep 2004; 50(9): 2882–2890.

30. Kiani AN, Johnson K, Chen C *et al.* Urine osteoprotegerin and monocyte chemoatractant protein-1 in lupus nephritis. J Rheumatol. 2007; 36(10):2224‐ 2230.

31. Gupta R, Yadav A, Aggarwal A. Longitudinal assessment of monocyte chemoattractant protein-1 in lupus nephritis as a biomarker of disease activity. Clin Rheumatol. 2016; 35:2707–2714.

32. Brunner HI, Bennett MR, Mina R *et al.* Association of noninvasively measured renal protein biomarkers with histologic features of lupus nephritis. Arthritis and Rheumatism. 2012; 64(8): 2687–2697.

33. Susianti, H. Iriane. V. M., Dharmanata, S. Handono, K., Widijanti, A., Gunawan, A., Kalim, H. Analysis of urinary TGF-1, MCP-1, NGAL and IL-17 as biomarkers for lupus nephritis. Pathophysiology.2015; 22: 65–71

34. Jason W, Bauer, Michelle Petri, Franak MB, Thearith Koeuth, Joseph Wilson, Catherine Slattery, Angela Panoskaltsis-Mortari, Peter K. Gregersen, Timothy W. Behrens, Emily C. Baechler. Interferon-Regulated Chemokines as Biomarkers of Systemic Lupus Erythematosus Disease Activity. Arthritis & Rheumatism. 2009; 60, (10): 3098-3107.

35. Lan L, Han F, Lang X, Chen J. 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. Biomarkers in Lupus Nephritis. 2016; 11(7):1-12.