Study of the Monocyte Chemoattractant Protein-1 (MCP-1) as a Biomarker of Lupus Nephritis Clinical Status

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Abstract: Background: SLE is an unpredictable multi-systemic autoimmune life-long disease whose etiology and pathogenesis are incompletely understood. The monocyte chomoattractant protein-1(MCP-1) is a chemokine thought to be responsible for monocyte and T lymphocytes recruitment in acute inflammatory conditions and may be an important mediator in chronic inflammation. **Objective:** Aimed to measure the serum and urinary levels of MCP-1 in patients with SLE, to find out its relation to clinical disease activity and to explore its role in LN. We also aimed to investigate correlations of these levels with histopathological changes of renal biopsies in order to evaluate their reliability as biomarkers for LN clinical status. **Patients and Methods:** After an ethical approval, this correlative study was conducted on thirty SLE female patients and 20 apparently healthy volunteers- age and sex matched. Serum and urinary levels of MCP-1 were determined by the ELISA technique, while a transcutaneous renal biopsy was obtained from all patients. **Results**: There were significant differences in the mean levels of serum MCP-1 (P<0.05) and urinary MCP-1 (P<0.001) between SLE patients and controls. The mean level of the urinary MCP-1 was significantly higher (P<0.001) in patients with active LN versus patients with inactive LN. There were insignificant differences (P>0.05) between the mean levels of C3 ,C4, ANA, anti-dsDNA and serum MCP-1 regarding the WHO classification system of LN, meanwhile, the mean urinary MCP-1 level showed a significant difference (P<0.05) among the histopathological groups. The diagnostic sensitivity and specificity of the urinary MPC-1 biomarker was greatest when it was combined with anti ds-DNA testing, being 88.8% sensitive and 100% specific. **Conclusion:** Urinary MCP-1 is a valuable, sensitive and non-invasive biomarker for the assessment of renal disease in SLE patients; it is well correlated with the clinical, laboratory and pathological parameters of LN activity. MCP-1 would be a potential therapeutic target in SLE.
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1. Introduction

SLE is an unpredictable multi-systemic autoimmune life-long disease whose etiology and pathogenesis are incompletely understood. Clinical features are diverse and episodic with differing immunological manifestations. Both adults and children with SLE have a significant morbidity and mortality, which is closely related to renal involvement [1]. Renal disease affects the great majority of patients with SLE (~ 66-90%) sometime at the evolution with lupus nephritis (LN) occurring in more than one-third of patients[2]. Production of nephritogenic autoantibodies, glomerular immune complex deposition and cytokine overproduction has been postulated to contribute to the pathogenesis of LN [3]. The presence of subendothelial deposits in glomerular capillaries is crucial for the induction of severe damage and it correlates with end-capillary proliferation, necrosis, karyorrhexis and crescent formation [2].

The monocyte chomoattractant protein-1(MCP-1) is a chemokine thought to be responsible for monocyte and T lymphocytes recruitment in acute inflammatory conditions and may be an important mediator in chronic inflammation [4]. The TNF superfamily cytokines-weak inducers of apoptosis (TWEAK) provoke the mesangial

cells, podocytes and endothelial cells to secrete pro-inflammatory chemokines including MCP-1, interleukin-10 which are crucial in the pathogenesis of LN[5]. Experimental data and studies on human renal tissue in patients with glomerulonephropathies indicated that MCP-1 plays a central role in progression of inflammatory processes in kidney diseases[6].

As LN is a major cause of morbidity and mortality in SLE, it is important to identify reliable, noninvasive methods to repeatedly assess the condition of the kidneys in these patients[5]. Moreover, treatment of SLE nephritis is associated with side effects that could be mitigated if the onset, severity and response of renal flare could be predicted, and therapy modified accordingly [7].

2- Patients and Methods

Thirty patients fulfilling at least four of the updated ACR revised criteria for the classification of SLE [8] were enrolled into this study. These patients were selected from the in-patients' and out-patients' clinics of the Rheumatology and Rehabilitation department of Benha University Hospitals through 2010. Twenty apparently healthy volunteers, age and sex matched to SLE patients, were carefully chosen as

a control group. This study was approved by the research ethical committee of our institution. Patients and controls were well educated about the study. They were clarified about objectives, importance, deliberate nature and procedures (including sequences and time they have to go through during the study). All patients were subjected to:

2.1-History taking:

Personal history, patients' complaint/s, history of present illness with stress on symptoms of renal disease, history of previous and present medications, past history and family history.

2.2-Clinical examination:

General examination, chest, cardiovascular, abdominal, neurological and musculoskeletal examinations.

2.3-Assessment of SLE disease activity:

According to Systemic Lupus Erythematosus Disease Activity Index [SLEDAI] score [9]. The renal SLEDAI (r SLEDAI) score consisted of 4 items of the total SLEDAI: hematuria, pyuria, proteinuria and urinary casts/HPF. The presence of each one of the 4 parameters takes a score of 4 points, thus the rSLEDAI score ranged from 0 to a maximum score of 16 [9]. Patients should have a negative urine bacterial culture and/or a negative CRP testing in the absence of antibiotic treatment to exclude the active urinary sediment of urinary tract infection. Patients were also excluded from the study, if they had diabetes or other inflammatory diseases.

2.4- Laboratory investigations:

Five centimeters of venous blood were obtained for the measurement of the erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), complete blood picture (CBC), renal function tests (serum creatinine, blood urea and creatinine clearance), antinuclear antibodies (ANA) titer, anti-dsDNA antibodies titer, complements (C3 and C4) levels and serum MCP-1. A fresh urine sample was obtained for complete urine analysis (urinary casts, hematuria, pyuria), urine protein: creatinine ratio and measurement of urinary MCP-1. Kits for serum and urinary of MCP-1 were obtained from **Bender Med systems GmbH, campus Vienna Biocenter 2, Austria, Europe** and levels were determined for all patients and control subjects by ELISA using a specific human monoclonal anti-human MCP-1 antibody according to Proost et al.[10].

2.5-Obtaining, staining and scoring the renal biopsy:

A renal biopsy from each patient was obtained on the same day of blood sampling under computed tomography "CT" guidance using a true cut needle biopsy. Specimens were fixed in 10% formalin solution, embedded in paraffin, sliced into 4 μm sections then stained with hematoxylin – eosin, for examination under light microscopy using the criteria of the World Health Organization (WHO) classification system for grading of LN [11].

The predominant histopathological class was reported for each sample. Activity and chronicity indices (AIS and CIS) respectively were used for biopsy assessment according to the standards of the National Institute of Health (NIH) for LN[12].

2.6-Ethical considerations:

Our study followed the principles of Helsinki Declaration developed by the World Medical Association (WMA). An informed consent was obtained from all participants. Only individuals who accepted to participate were included in the study. The consent included data about objectives of the work. Participants were informed that all data are collected for scientific research only and will be confidential. No harmful techniques or interventions will be applied.

2.7-Statistical analysis:

Results of this study were tabulated and statistically analyzed by descriptive and analytic statistics on IBM personal computer using the SPSS version 6. For groups having quantitative variables with different variance, statistical results were further analyzed by Dunns' post hoc testing.

3-Results

This study included 30 patients with SLE. They were all females (100%) with a mean age of 27.7±8.2 years and disease duration of 4.2 ± 3 years. Patients and controls were matched for age (P>0.05) and sex (P>0.05). After proper examinations, SLE patients were stratified to one of 2 groups:

Group I: Included 6 female SLE patients (20%), who had never shown any past or present clinical and/or laboratory evidence of major renal manifestation attributable to SLE as well as having normal renal biopsies. Their mean age was 29.67 ± 8.3 years while, their mean disease duration was 2 ± 1.8 years.

Group II: Included 24 female SLE patients (80%) with renal disease based on the results of the renal biopsy. Their mean age was 28.7± 8.7 years while; their mean disease duration was 4.8 ± 3.1 years.

There was a significant difference between group I and II regarding their disease duration (P<0.05). **Group II was subdivided into 2 subgroups:**

Group II A: including 18 female patients (75%) with active LN (rSLEDAI score ≥ 4) and **Group II B:** including 6 female patients (25%) with inactive renal disease (rSLEDAI score = 0).

Renal SLE disease affected 80% (24/30) of our patients, with 75% of them (18/24) having *active* LN, while 25% (6/24) of them had *inactive* LN. The prevalence of *active LN* in all SLE patients was 60% (18/30).

Table (1): Comparisons of MCP-1 levels among the studied groups

Group / Variable	*Serum* MCP-1 (pg/ml) Mean ± SD	*Urinary* MCP-1 (pg/ml) Mean ± SD
**All SLE patients	192.7±54.5	1790.5 ±874.2
Group I ($n = 6$)	187.7±33.2	700±89.4
Group II ($n = 24$)	194±59.1	2063.2±757.6
Controls	150.8±68.2	399.3±85.6
F	2.3	56.5
P	< 0.05*	< 0.001*

*Significant if ($P < 0.05$).
** There were statistically significant differences between n the mean levels of either *serum* MCP-1 (P <0.05) or *urinary* MCP-1 (P <0.001) of SLE patients and the control group.

- Post-hoc testing, revealed that there was statistically significant difference in the mean level of the *serum* MCP-1 between group II and the control group (P <0.05), while there were insignificant differences between group I and group II as well as group I and the control group (P>0.05).

- The mean level of *urinary* MCP-1 was statistically significantly higher in group II than group I and the control group (**P** < 0.001), while there was insignificant difference in the mean level of *urinary* MCP-1 between group I and the control group (**P** > 0.05)

Table (2): Comparisons of laboratory parameters among the studied SLE patients' groups

Group / Variable	Group I (n=6) Mean ± SD	Group IIA (n=18) Mean ± SD	Group IIB (n=6) Mean ± SD	F	P
ESR mm / 1st hr	42.3±6.8	71.9±18.01	50±15.5	9.2	<0.05*
Creatinine clearance ml/min	110±8.9	60.4±10.01	75±4.5	10.53	<0.05*
S. creatinine mg/dl	0.97±0.19	1.4±0.5	0.8±0.2	4.9	<0.05*
S. urea mg/dl	11.7±6.8	23.9±7.3	16.3±3.7	8.5	>0.05
Urine ptn. to creatinine ratio	0.133± 0.086	1.634±1.081	0.358±0.056	9.5	<0.05*
C3 mg/dl	130.7±15.00	87.8±7.9	100.7±15.00	33.7	<0.05*
C4 mg/dl	31±9.1	14.8±4.7	25±11.8	12.4	>0.05
ANA U/ml	104.7 ±29.2	120 ±86.5	94.7 ±19.1	1.02	>0.05
Anti -ds DNA U/ml	22.7±8.5	109± 25.5	97.4±35.02	11.03	<0.05*
Serum MCP-1 pg/ml	187.7±33.2	199.2±64.6	178.5±38.7	2.3	>0.05
Urinary MCP-1 pg/ml	700±89.4	2409.8±516.3	1023.3±60.9	56.5	<0.001*

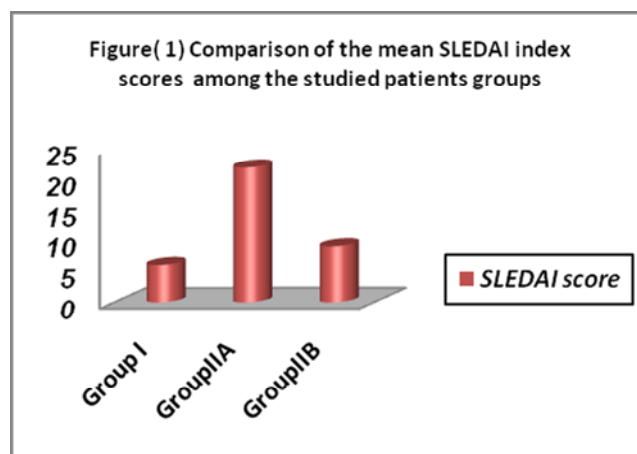*Significant if ($P < 0.05$).

Table (3): Histopathological grading of renal biopsies of LN patients

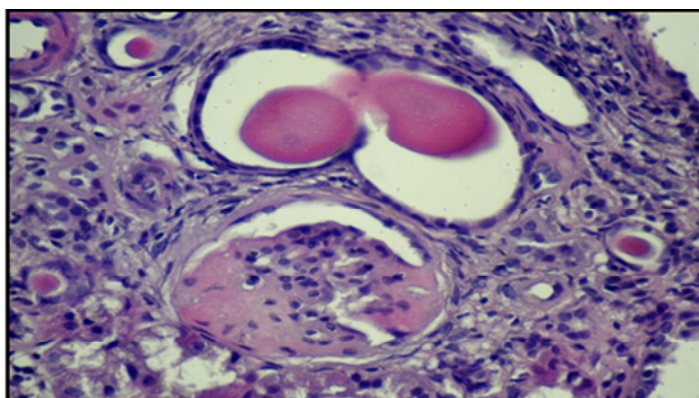| Grade / Frequency	II	III	IV
$n = 24$ (100%)	10(42%)	8 (33%)	6 (25%)

- Post-hoc testing, revealed statistically significant difference among SLE patients' groups as regards the mean value of ESR (P<0.05) being **higher** in group IIA, creatinine clearance (P <0.05) being **lower** in group IIA, urine protein to creatinine ratio (P <0.05) being **higher** in group IIA and the C3 level (P <0.05) being **lower** in group IIA.

- There was insignificant difference (P >0.05) between the mean level of *serum* MCP-1 in group IIA (active LN patients) and group IIB inactive LN patients.
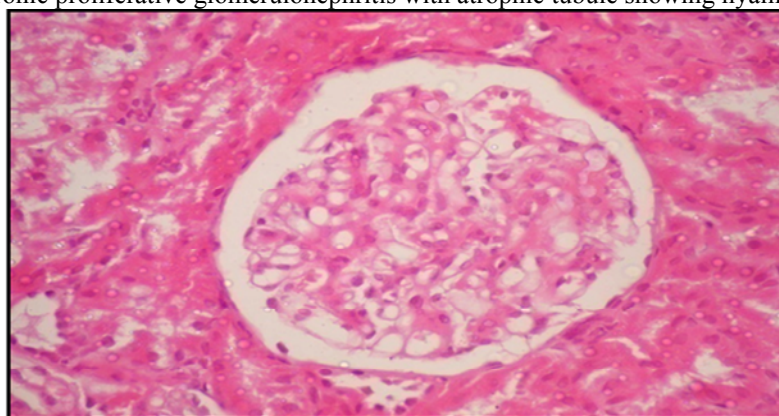- There was highly statistically significant difference (P <0.001) between the mean value of *urinary* MCP-1 in group IIA (active LN patients) and group IIB (inactive LN patients).

Figure(1) Comparison of the mean SLEDAI index scores among the studied patients groups

Post-hoc testing, revealed statistically significant increase in the mean SLEDAI score of group IIA versus group IIB and I (P <0.05), with an insignificant difference in the mean SLEDAI score between group IIB and group I (P >0.05).
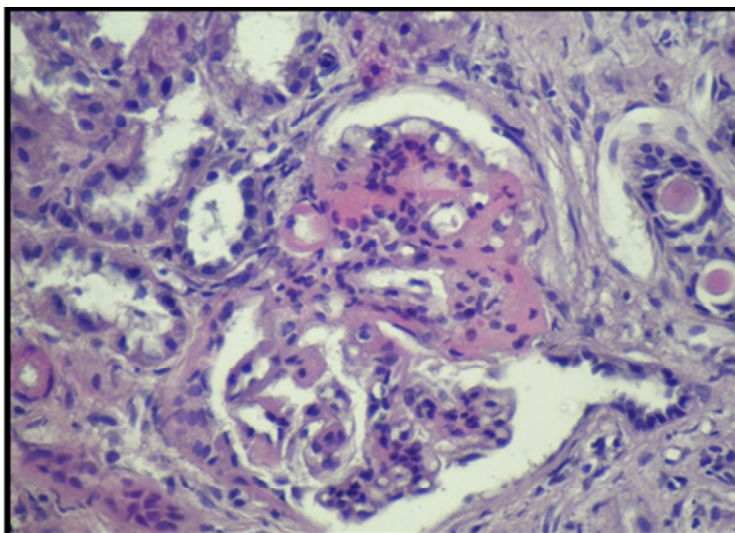

Figure (2): Chronic proliferative glomerulonephritis with atrophic tubule showing hyaline casts *(H&E, X400).*

Figure (3): Glomerulonephritis class (III) with fibrinoid necrosis. The necrotizing lesions are associated with a clinical course of severe renal involvement and with greater probability of chronic glomerular changes. *(H&E, X400).*

Figure (4): Focal proliferative GN class III with segmental glomerular sclerosis *(H&E X400).*

Table (4): Pathological activity and chronicity indices scores in the LN patients' groups

Group / Variable	Group IIA	Group IIB
Activity index score (max =24) Range mean±SD	8-20 13.55 ± 4.36	1-7 3.66 ± 3.06
Chronicity index score (max =12) Range mean±SD	1-7 3.67 ± 2.18	3-5 3.67 ± 3.33

Table (5): Comparison among laboratory parameters as regards histopathology
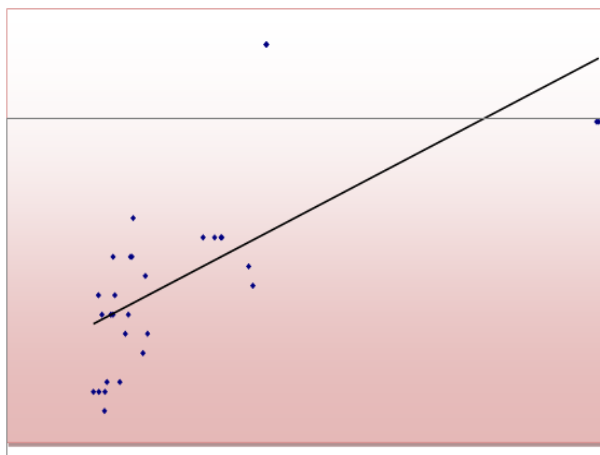
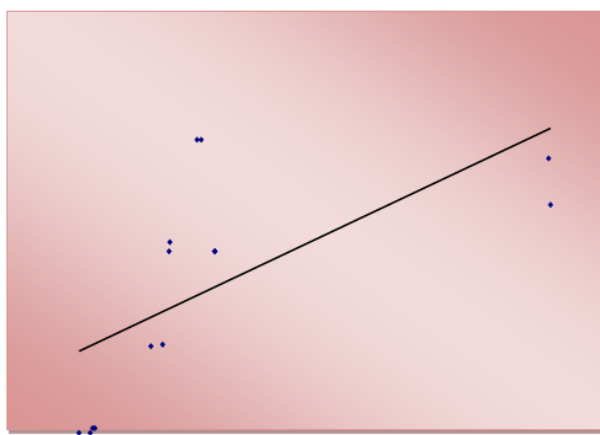| Class / Variable	Class II n = 10 Mean ± SD	Class III n = 8 Mean ± SD	Class IV n = 6 Mean ± SD	F	P
C3 mg/dl	89.2 ± 1.92	95± 16.64	88.333 ± 16.07	3.05	>0.05
C4 mg/dl	17.8 ± 9.17	21± 9.7	11.66 ± 1.53	1.86	>0.05
ANA U/ml	101.8 ± 18.23	78.25± 29.84	181 ± 97.34	2.41	>0.05
Anti -ds DNA U/ml	85.8 ± 21.04	126.75± 21	89.33 ± 50.24	3.58	>0.05
Serum MCP-1 pg/ml	179.3±48.1	189.5±51.1	224.5±81.8	1.1	> 0.05
Urinary MCP-1 Pg/ml	1517.6±417.3	2042.5±679.01	3000±178.9	17.5	< 0.05*

*Significant if ($P < 0.05$)

- Regarding *urinary* MCP-1 level, there was a statistically significant difference ($P<0.05$)* between groups, being **higher** in patients with class IV-and **lower** in patients with class II.
- Regarding the clinical features in SLE patients, the mean *serum* MCP-1 level was significantly higher in the presence arthritis/arthralgia ($P <0.05$).
- The mean *urinary* MCP-1 level was significantly higher in patients with renal affection ($P <0.001$).
- There were statistically insignificant correlations ($P>0.05$) between *serum* MCP-1 levels and all laboratory markers of SLE as well as SLEDAI score ($r = 0.06$) or rSLEDAI score ($r = 0.14$).
- There were statistically significant positive correlations between the *urinary* MCP-1 levels and ESR ($r = 0.89$), anti-ds DNA titre ($r = 0.44$), protein:creatinine ratio ($r = 0.89$, $P < 0.001$), SLEDAI score ($r = 0.91$ rSLEDAI score and ($r= 0.95$). In addition, there were statistically significant negative correlations with HB % ($r = -0.68$), creatinine clearance ($r = - 0.89$) and C3 level ($r = - 0.28$).

Figure (5): Positive Correlation between the *urinary* MCP-1 levels and urine protein: creatinine ratio.

Figure (6): Positive Correlation of *urinary* MCP-1 levels with the rSLEDAI scores

Table (6): Correlations of laboratory parameters regarding activity and chronicity scores of renal biopsy in SLE patients

	Activity index scores		Chronicity index Scores	
	r	P	*R*	*P*
C3 mg/dl	0.15	> 0.05	2.98	> 0.05
C4 mg/dl	2.87	> 0.05	1.43	> 0.05
ANA u/ml	0.24	> 0.05	1.84	> 0.05
Anti-ds DNA u/ml	1.78	> 0.05	0.56	> 0.05
Serum MCP-1 pg/ml	0.14	> 0.05	0.18	> 0.05
Urinary MCP-1 Pg/ml	0.38	< 0.05*	0.02	> 0.05

*Significant if ($P < 0.05$).   *r* = Spearman's correlation.
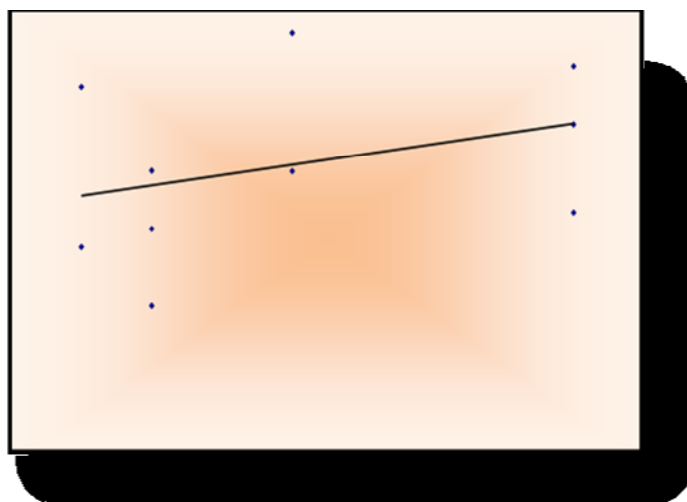
 - There was only a statistically significant positive correlation ($P < 0.05$) between *urinary* MCP-1 levels and the activity scores (*r* = 0.38) of the examined renal biopsies.

Table (7): Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPP) of anti-ds DNA antibody, *serum* and *urinary* MCP-1 in active LN

	Sensitivity	Specificity	PPV	NPV
Anti–ds DNA antibody	88.8%	83.3%	76.1%	33.3%
Serum **MCP-1**	33.3%	33.3%	60%	8.3%
Urinary **MCP1**	94.4%	83.3%	89.4%	80%
Urinary **MCP-1& anti-ds DNA**	88.8%	100%	88.8%	25%

Figure (7): Correlation between *urinary* MCP-1 levels and the activity scores of the renal biopsies in LN patients (Group II)

Sixteen patients in group IIA (88.8%) were found positive for both the anti-ds DNA antibody and the *urinary* MCP-1, while none of the patients in group IIB were found positive for both anti-ds DNA and the *urinary* MCP-1. This yielded a diagnostic sensitivity of 88.8%, specificity of 100%, PPV of 88.8% and NPV of 25% for both the anti-ds DNA antibody and the *urinary* MCP-1 in LN.

Urinary MCP -1 showed a higher diagnostic sensitivity of 94.4% and equal specificity of 83.3% to the anti-ds DNA antibody alone. The diagnostic sensitivity and specificity were greatest when both tests were found positive in combination being 88.8% for the diagnostic sensitivity and 100% for the diagnostic specificity

4-Discussion

SLE is a chronic, relapsing inflammation and often febrile multi – systemic disorder of connective tissue, characterized principally by involvement of the skin, joints, kidney and serosal membranes. It is of unknown etiology, but is thought to represent a failure of the regulatory mechanisms of the autoimmune system [13].

Conventional clinical parameters such as creatinine clearance, proteinuria, urine sediments, anti-dsDNA, and complement levels are not sensitive or specific enough for detecting ongoing disease activity in the lupus kidneys and early relapse of nephritis [14]

Renal biopsy is the gold standard for providing information on the histological classes of LN and the relative degree of activity and chronicity in the glomeruli. However, it is invasive and serial biopsies are impractical in the monitoring of LN. Thus, novel biomarkers that are able to discriminate lupus renal activity and its severity, predict renal flares, and monitor treatment response and disease progress are clearly necessary [15].

It has been well established that proinflammatory chemokines play a critical role in the pathogenesis of experimental SLE nephritis (and the presence of chemokines in the urine of patients with SLE nephritis reflects intra- renal chemokine expression [16].

In this study we found that renal affection (clinically and histopathologically) was present in 80% of patients with SLE, similar to *Kim et al.* [17], who stated that renal involvement is clinically evident in 40% to 85% of SLE patients.

This was lower than the result of *Gigante et al.* [18], who found that renal affection occurred in about 60% of their patients. *Galleli et al.* [19], also reported that the incidence of LN was 50% in 60 patients.

The higher percentage in our study may be attributed to the diagnosis of LN based the results of the renal biopsy.

In our study, serum MCP-1 levels were significantly high (P <0.05) in SLE patients (192.7±54.5 pg/ml) compared to the controls (150.8± 68.2 pg/ml). These levels were even significantly higher in patients with LN (194±59.1 pg/ml) than the controls (P <0.05). Insignificant difference (P>0.05) was found between patients without nephritis (187.7±33.2 pg/ml) and either those with nephritis or the controls. These results were also in agreement with *Alzawawy et al.*[20].

Lit et al. [21], found that the plasma concentration of inflammatory cytokines including MCP-1 were significantly higher in all SLE patients than healthy individuals. However, when their patient's cohort was stratified into renal involvement subgroups analysis, patients with renal involvement had a lower plasma concentration of MCP-1. They suggested that urine chemokines could serve as biomarkers for renal disease flare and that the lack

of significant increase in the circulating levels of serum MCP-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 MCP-1 in the serum.

Urinary MCP-1 levels, were significantly higher (P <0.001) in our SLE patients and in those with nephritis (P <0.05) than controls, while, they were insignificantly different between those without LN and 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.* [20] *and Tucci et al.* [3].

This study revealed that there was insignificant statistical difference (P>0.05) in the mean serum MCP-1 level between patients with active nephritis and those with inactive nephritis. This difference was significantly high (P <0.001) regarding the mean urinary MCP-1 level.

Rovin et al. [22], obtained serial measurement of urine chemokines in a cohort of prospectively followed SLE patients and found that urinary MCP-1 increases significantly at renal flare. They also reported changes of the urinary MCP-1 overtime paralleled the onset and the resolution of flare. Non-renal SLE flares were not accompanied by increase in urinary MCP-1 indicating that urine chemokines do not reflect general systemic SLE activity.

Our study showed that there were insignificant correlations of serum MCP-1 levels with all laboratory parameters of SLE.

As regards to urinary MCP-1 levels, we found them correlated positively with the anti-dsDNA antibody titer (r = 0.44, P < 0.05) and negatively with C3 (r = -0.81, P< 0.05). This finding was similar to those found by *Kiani et al.* [23], *Tucci et al.* [3] *and Jason et al.* [24].

Our study showed a highly significant positive correlation of urinary MCP-1with rSLEDAI score (P<0.001) and insignificant correlation with the total SLEDAI score (P >0.05). This emphasized that elevations of urinary MCP-1 in SLE patients were specific to renal activity.

In addition, *Kiani et al.* [23], showed that urinary MCP-1 was associated with measures of disease activity including patient's Global assessment (PGA), and the SLEDAI using SLEDAI renal descriptors.

Chan et al. [25], stated that the urinary MCP-1 correlated with the degree of proteinuria (r =0.73, P <0.001) and serum creatinine level (r =0.45 P < 0.001).

We found insignificant correlation of urinary MCP-1 with serum MCP-1 levels. However, *Tucci et al.* [3], indicated that urinary MCP-1 levels reflect predominantly local production of this chemokine rather than simple filtration.

Urinary MCP-1 levels, varied significantly (P < 0.05) among the histopathological groups. *Seshan and Chales* [26], explained the difference in levels of urinary MCP-1 among patients with WHO class IV, V and II by decreased cellularity in glomerular and tubulo-interstitial lesions in WHO class I and class II than WHO class IV.

On the other hand, there was statistically significant positive correlation (r = 0.38; P <0.05) of urinary MCP-1 levels with the activity scores of the examined renal biopsies, while there was insignificant correlation (r = 0.02; P >0.05) with the chronicity scores. This confirmed the findings of *Tucci et al.* [3]. These results further support the notion that MCP-1 may contribute to the development of renal lesions.

Marks et al. [27], stated that genetic polymorphism of MCP-1 gene may predispose to the development of SLE and found that certain genotypes are at higher risk of developing LN through modulating MCP-1 expression. This would suggest its possible role in etiopathogenesis of LN with the useful monitoring of MCP-1 as a marker of disease activity.

Conclusion

Urinary MCP-1 is a valuable, sensitive and non-invasive biomarker for the assessment of LN in SLE patients. It is well correlated with the clinical, laboratory and pathological parameters of LN activity. MCP-1 would be a potential therapeutic target in SLE.

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