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Detection of Urinary CD4TCell in Patients with Lupus Nephritis during Activity and Treatment period

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ABSTRACT

Background: Renal involvement is common in Systemic Lupus Erythromatosis (SLE) and is a significant cause of morbidity and mortality. It is estimated that as many as 90% of patients with SLE will have pathologic evidence of renal involvement on biopsy, but clinically significant nephritis will develop in only 50%. The clinical presentation of lupus nephritis is highly variable, ranging from asymptomatic hematuria and/or proteinuria, to frank nephrotic syndrome, to rapidly progressive glomerulonephritis with loss of renal function. Lupus nephritis typically develops within the first 36 months of the disease, although there are exceptions.

Aim of the Work: The aim of this work was to study the level of urinary CD4 T cell and monitor the treatment in lupus nephritis patients.

Patients and Methods: The ethical approval was obtained from the hospital ethical research committee and each patient participated in the study signed an informed consent. The present study was conducted on seventy-five female subjects, their age ranged from 20 to 40 years. They were divided into 3 groups a- 25 patients knowns as SLE with lupus nephritis, b- 25 SLE patients without lupus nephritis, c-25 normal control subjects. They were recruited from Physical Medicine, Rheumatology and Rehabilitation department of Sayed Jalal and Al-Hussein Al-Azhar University Hospitals, during the period from October 2013 to October 2017.

Results: Urinary CD4 markedly decreased after treatment of lupus nephritis.

Conclusion: Urinary CD4 T-cells marker has a valuable role in detecting LN in SLE patients and has a significant correlation with disease activity index. Significant Positive correlation was found between Cd4 t-cell and SLEDAI in before and after treatment. Significant positive correlation was detected with 24hr urine protein, SLEDAI, PGA, while Platelet, ESR and C3 significant and negative correlation in post treatment. Monitoring urinary CD4 T-cells may help to identify treatment responders and treatment failure and enable patient-tailored therapy in the future.

Keywords: Lupus nephritis, CD4

INTRODUCTION

Renal involvement is common in Systemic Lupus Erythromatosis (SLE) and is a significant cause of morbidity and mortality. It is estimated that as many as 90% of patients with SLE will have pathologic evidence of renal involvement on biopsy, but clinically significant nephritis will develop in only 50%. The clinical presentation of lupus nephritis is highly variable, ranging from asymptomatic hematuria and/or proteinuria, to frank nephrotic syndrome, to rapidly progressive glomerulonephritis with loss of renal function. Lupus nephritis typically develops within the first 36 months of the disease, although there are exceptions [(1,2)].

In lupus nephritis, the infiltrates in the nephritic kidneys consist mainly of CD4 T cells and, to a lesser extent, CD8 T cells, macrophages cells, and plasma cells [(3)].

In severe lupus nephritis, as in other forms of rapidly progressive glomerulonephritis, CD4 + T cells are present within glomeruli [(4)].

AIM OF THE WORK

The aim of this work was to study the level of urinary CD4 T cell and monitor the treatment in lupus nephritis patients.

PATIENTS AND METHODS

The present study was conducted on seventy-five female subjects, their age ranged from 20 to 40 years. They were divided into 3 groups a- 25 patients knowns as SLE with lupus nephritis, b- 25 SLE patients without lupus nephritis, c-25 normal control subjects. They were recruited from Physical Medicine, Rheumatology and Rehabilitation department of Sayed Jalal and Al-Hussein Al-Azhar University Hospitals, during the period from October 2013 to October 2017.

An approval was obtained from the medical ethics committee of Al-Azhar University before starting this study. All the patients were informed about the study procedures and a written consent was obtained from all of them.

The subjects were divided into three groups: Group A: 25 SLE patients with lupus nephritis. Group B: 25 SLE patients without lupus nephritis. Group C: 25 healthy control subjects

Patient's selection

A) Inclusion criteria for group A &B:

Patients were fulfilled the revised ACR and the SLICC classification criteria for SLE (2015).

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All Patients were females. Age of patients ranged from 20 to 40 years old. Patients with disease duration (3-10) years were included in the present study. Established diagnosis of lupus nephritis by renal biopsy.

B) Exclusion criteria:

Patients suffered from any chronic debilitating diseases (DM, TB …… etc.). Recent sudden or marked change in body weight (for known or unknown causes). Associated rheumatic disorders (Vasculitis or amyloidosis….. etc.). Pregnancy or lactation.

I-Full medical history taking:

Personal History about, Name, Age, Sex, Residence, Occupation, Marital status, Special Habits of medical importance, in addition to Menstrual history and Obstetric history including number of labors and abortions for female patients.

History of present illness: Onset, course and disease duration. Constitutional symptoms as fatigue, fever and loss of weight. Musculoskeletal manifestations as morning stiffness, joint pain, swelling, redness, hotness and muscle pain or weakness. Mucocutaneous manifestations as rash, photosensitivity, alopecia, oral ulcers, Raynaud's phenomena, skin ulcers and splinter hemorrhage. Gastrointestinal manifestations as anorexia, epigastric pain, nausea, vomiting, diarrhea and constipation. Renal manifestations as loin pain, hematuria, dysuria, urgency, frequency, polyuria or oliguria, puffiness of the eye lids and swelling of the lower limbs.

Past history: Drug therapy: steroids, immunosuppressive therapy (doses and duration of therapy) and drug induced lupus. Previous operations or hospitalization. Drug allergy. History of blood transfusion.

Family history: Family History of SLE cases or other autoimmune diseases.

II- Examination: General examination. Examination of Scalp, Eye and Skin. Cardiac examination. Chest examination. Abdominal examination. Neuropsychiatric examination. Articular examination, all joints were examined as follow: Inspection: overlying skin color, muscle wasting, deformity and swelling. Palpation: temperature, tenderness and swelling (soft tissue or effusion). Movement: both active and passive movement was done, with observation of pain, crepitus and protecting muscle spasm.

III-Assessment of the disease activity using:

The Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score. **Content:** Specific manifestations in 9 organs/systems Number of items: 24 items covering 9 organs systems.

Response options/scale: There are 24 items for the 9 organs/systems. Scored if present within the last 10 days. Two systems can score a maximum of 8 points each, 2 systems can score a maximum of 4 points each, 3 systems can score a maximum of 2 points each, and 2 systems can score a maximum of 1 point each. Scores range from 0–105 points.

Recall period for items: Within the last 10 days. Recently, the SLEDAI-2K for a time frame of 30 days prior to a visit for clinical and laboratory variables was shown to be similar to the SLEDAI-2K for 10 days.

Score Interpretation: The score range is 0–105 points. A score of 6 is considered clinically important and affects decision to treat (5).

Disease activity and renal disease activity were assessed by the SLE Disease Activity Index 2000 update (SLEDAI-2K) and renal SLEDAI (rSLEDAI), respectively. The rSLEDAI (SLEDAI-2K renal scores) comprised haematuria, proteinuria, pyuria and urinary casts. LN patients were divided into two groups according to SLEDAI scores, active LN (SLEDAI ≥8) and inactive LN (SLEDAI <8).

Investigations: Complete Blood Picture (CBC): analyzed using coulter counter (T660).

C-Reactive protein (CRP): (assay by Bio-Med-CRP latex agglutination method).

Erythrocyte sedimentation rate (ESR): by Westergren method. The reading of first hour was taken.

Liver function tests: Serum ALT, AST by Hitachi Cobas C 311 automated analyzer.

Renal function tests: Serum Creatinine, Blood Urea and Serum Uric acid by Hitachi Cobas C 311 automated analyzer.

Blood sugar: Fasting Blood Glucose and 2-Hours Post Prandial Blood Glucose Level by Hitachi Cobas C 311 automated analyzer. Urine

analysis. 24-hour protein/ in urine: 24-hour urine and spot urine sample was collected to estimate urinary protein excretion.

Autoimmune profile: Including Complement C3, C4 and Anti.double-strand -DNA Antibody and anti-smith Ab was detected by an enzyme-linked immune-sorbent assay (ELISA).

Urinary CD4 T cell:

The procedure:

Urine samples collected from 50 SLE patients and 25 healthy controls were monitored for the presence of CD4T-cells at baseline and repeated measurements after 6 months were done for the patients only. The median sample size was 100 ml urine; the usual standing time for the urine was 4-6 hours.

The CD4 antigen is involved in the recognition of MHC class II molecules. CD4 is primarily expressed in a subset of T-lymphocytes, also referred to as T helper cells, but may also be expressed by other cells in the immune system, such as monocytes, macrophages, and dendritic cells.

At the tissue level, CD4 expression may be detected in thymus, lymph nodes, tonsils, and spleen, and also in specific regions of the brain, gut, and other non-lymphoid tissues.

CD4 functions to initiate or augment the early phase of T-cell activation through its association with the T-cell receptor complex and protein tyrosine kinase. It may also function as an important mediator of direct neuronal damage in infectious and immune-mediated diseases of the central nervous system. Multiple alternatively spliced transcripts have been identified in this gene.

Flow cytometry method: In this prescriptive study 4 colors was used to quantify the percent and events of CD4 in the monoclonal cells of urine sample, so for assessment we used CD4 PE, CD3 Prep, CD45 FITC and the urine sample were collected immediately, centrifuged and washed 3 times with phosphate buffer solution (PBS), then we incubate the pellet with monoclonal Abs for assessment with flow cytometry, also we added PromodiumIodid (Eugene,Oregon, USA) immediately before assay on flow cytometry to exclude dead cells.

For each sample up to 10.000 cells were acquired and according to the plot of Side Scatter Scale versus CD45 stain our data was visualized. Lymphocyte populations were identified based on low Side Scatter and a combination of low intensity CD45 expression with CD4 CD3.

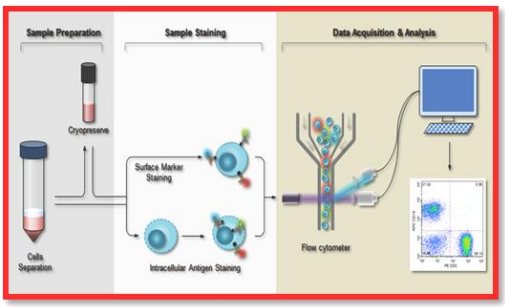

Figure (1): Flow Cytometry (FCM) /FACS Protocol

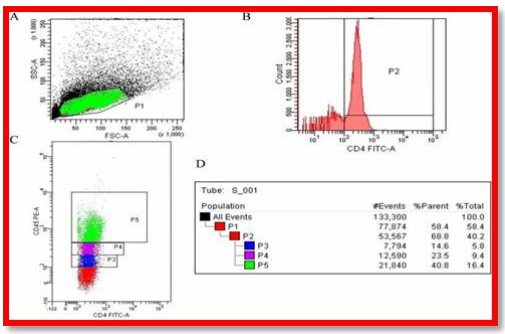

Figure (2): Diagrams illustrate CD4 FITC detection.

Follow-up: After six months urinary CD4 significantly decreased after treatment of lupus nephritis.

Statistical analysis: Recorded data were analyzed using the statistical package for social sciences, version 20.0 (SPSS Inc., Chicago, Illinois, USA). Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage.

The following tests were done: Independent-samples t-test of significance was used when comparing between two means. Paired sample t-test of significance was used when comparing between related sample. A one-way analysis of variance (ANOVA) when comparing between more than two means. Chi-square (x2) test of significance was used in order to compare proportions between two qualitative parameters. Pearson's correlation coefficient (r) test was used to assess the degree of association between two sets of variables.

The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as the following: Probability (P-value). P-value <0.05 was considered significant. P-value <0.001 was considered as highly significant. P-value >0.05 was considered insignificant.

RESULTS

Table (1): Comparison between groups according to demographic data.

Demographic data	Group A: SLE with nephritis (N=25)	Group B: SLE without nephritis (N=25)	Group C: Normal person (N=25)	F/x2#	p-value
Age (years) Mean ± SD	30.40±8.11	28.92±7.58	29.15±6.97	1.691	0.408
Range	20-40	20-40	20-40		
Sex Female	25 (100%)	25 (100%)	25 (100%)	0.000#	1.000

I-Demographic data:

According to the demographic data; for the three group A, B and C, all subjects were Egyptian females with age ranged from 20 to 40 years old.

For group A &B; patients with established SLE diagnosis according to the revised ACR and the SLICC classification criteria for SLE (2015). Disease duration ranged from 3 to

10 years with mean 6±4.3

As regard the comparison between the three groups according to demographic data, there was no statistically significant difference between them.

Table (2): Comparison between groups according to SLEDAI and PGA.

	Group A: SLE with nephritis (N=25)	Group B: SLE without nephritis (N=25)	t-test	p-value
BILAG Mean±SD Range	3.4±0.68 3-9	1±0.870 0-3	67.57	<0.001**
SLEDAI Mean±SD Range	17.84±7.69 8-34	5.80±1.68 2-9	58.477	<0.001**
PGA Mean±SD Range	2.40±0.58 1-3	1.32±0.90 0-3	25.504	<0.001**

II-Clinical data in the form of (Assessment of disease activity by BILAG,SLEDAI and PGA: Disease activity was evaluated for patients of both group A &B by SLEDAI and PGA. Group (A) revealed high scores of SLEDAI ranged from 8 to 34 with mean 17.84±7.69, while group (B) showed low scores ranged from 2-9 with mean 5.80±1.68.

Group (A) revealed high scores of PGA ranged from 1 to 3 with mean 2.40±0.58, while group (B) showed low scores ranged from 0-3 with mean 1.32±0.90.

As regard the comparison between the groups A&B according to BILAG, SLEDAI & PGA, there was highly statistically significant difference between groups

Table (3): Comparison between groups according to C3 and C4.

	Group A: SLE with nephritis (N=25)	Group B: SLE without nephritis (N=25)	t-test	p-value
C3 (mg/dl) Mean±SD	64.10±14.50	91.02±15.03	6.318	0.015*
C4 (mg/dl) Mean±SD	35.71±25.18	47.50±21.05	3.924	0.039*

The C3 serum level for patients of Group (A) was ranged from 20 to 190 with mean 64.10±40.50, while C4 serum level ranged from 1.9-96 with mean 35.71±25.18.

The C3 serum level for patients of Group (B) was ranged from 37 to 196 with mean 91.02±35.03, while C4 serum level ranged from 5.7-96 with mean 47.50±21.05.

Comparison between the two group according to C3 and C4 shows statistically significant difference between groups.

Table (4): Comparison between groups according to pre CD4 t-cell.

Pre Cd4 t Cell	Group A: SLE with nephritis (N=25)	Group B: SLE without nephritis (N=25)	Group C: Normal person (N=25)	ANOVA	p-value
Mean±SD	2624.00±1091.46	216.36±51.73	2.47±0.22	121.377	<0.001**
Range	1300-5000	153-333	0-5		

F: ANOVA; p-value <0.001** HS

Group A: Showed highly elevated urinary CD4 t-cell level with range 1300-5000 and mean 2624.00±1091.46.

Group B: Showed urinary CD4 t-cell level with range 153333 and mean 216.36±51.73.

Group C: insignificant levels.

Comparison between the two group according to CD4 t-cell showed statistically significant difference between groups.

Table (5): Comparison between before and after treatment according to CD4 t cell in group A: SLE with nephritis.

CD4 T cell	Mean	±SD	Mean Diff.	Paired Sample ttest	p-value
before treatment	2624.00	191.46	1024.00	10.859	<0.001**
After treatment	1600.00	91.77			

p-value<0.001** HS

Post management CD4 T-cell urinary level was assessed and showed marked improvement with highly statistically significant difference between pre and post according to CD4T-cell.

Table (6): Correlation between CD4 T cell and other parameters, using Pearson Correlation Coefficient in group A: SLE with nephritis.

Group A: SLE with nephritis		CD4 T cell		
	Before treatment		After treatment	
	r	p-value	r	p-value
Age (years)	-0.317	0.123	-0.288	0.163
WBC ($\times 10^3$)	-0.091	0.666	-0.233	0.261
Lymphocyte ($\times 10^3$)	-0.100	0.634	-0.142	0.500
Hemoglobin (g/L)	-0.037	0.860	-0.096	0.649
Platelet ($\times 10^3$)	-0.367	0.071	-0.466	0.019*
24hr urine protein (g/d)	0.356	0.081	0.551	0.004*
Serum creatinine (mg/dl)	-0.066	0.753	0.282	0.171
Serum uric acid (mg/dl)	-0.195	0.352	0.128	0.543
B. urea (mg/dl)	-0.029	0.892	-0.076	0.720
ESR (mg/dl)	-0.146	0.485	-0.448	0.025*
CRP (mg/L)	-0.074	0.727	-0.005	0.982
ds-DNA (IU/L)	-0.366	0.072	-0.139	0.506
C3 (mg/dl)	-0.130	0.536	-0.348	0.023*
C4 (mg/dl)	0.272	0.188	0.191	0.360
SLEDAI	0.566	0.003*	0.807	<0.001**
PGA	0.374	0.065	0.585	0.002*

r- Pearson Correlation Coefficient

*p-value <0.05 S; p-value <0.001** HS; p-value >0.05 NS

Correlation between CD4 T cell and other parameters, using Pearson Correlation Coefficient in group A: SLE with nephritis showed:

Positive correlation and significant between CD4T-cell and SLEDAI in pretreatment.

Also, significant positive correlation with 24hr urine protein, SLEDAI, PGA, while Platelet, ESR and C3showed significant and negative correlation in post treatment.

DISCUSSION

In a study done by Moon et al.they indicated that intra-renal infiltration and activation of T-cells in the interstitium is the main mechanism of kidney injury and showed a predominant CD4 T-cell renal infiltration [(6)].

Another study reported that in LN the interstitial infiltration consists mainly out-cells and to lesser extent of macrophages, B-cells and plasma cells [(7)].

Enghard et al. determined whether the infiltrating T cells could be monitored in the urine to provide a reliable biomarker for acute LN. The frequency of CD4 T-cells was determined by flow cytometry of peripheral blood and urine from 38 patients with SLE and the values were compared with disease activity as determined by the SLEDAI. The number of urinary CD4 T-cells reflected nephritis activity and elevation above 800 CD4 T-cells per 100 ml of urine sharply delineated active from inactive nephritis. Moreover, the frequency of urinary CD4 T-cells correlated with the disease activity [(8)].

In our current study, 50 SLE patients were monitored as follow-up, 25 patients with nephritis and 25 without nephritis and 25 healthy volunteers were included as controls.

It was found that, there was highly statistically significant difference between groups according to pre CD4 T-cell.(before treatment), And also after 6 months of treatment and follow up.

Moreover, There was Positive correlation and significant between Cd4 t-cell and SLEDAI in pretreatment and there was significant positive correlation with 24hr urine protein, SLEDAI, PGA, in post treatment.

In the view of the abovementioned information, our result indicated that Urinary CD4 T-cells marker has a valuable role in detecting LN in SLE patients and has significant correlation with disease activity index.

And this results are in accordance with ***Enghard*** [(9)] who measured the level of urinary CD3CD4 T-cells using flow-cytometry in 186 urine samples from 147 patients with SLE. Fourteen patients were monitored as follow-up. Thirty-one patients with other nephropathies and 20 healthy volunteers were included as controls. In SLE, urinary CD4 T- cell counts ≥800/100 ml were observed exclusively in patients with active LN. In patients monitored under therapy, normalization of urinary CD4 T-cell counts indicated lower disease activity and better renal function. In contrast, patients with persistence of, or increase in, urinary T-cells displayed worse outcomes.

Our results agreed also with ***Kopetschkeand*** [(10)] who studied 98 patients with SLE, 19 with active renal involvement defined by (SLEDAI ≥ 10& a current renal biopsy showed LN or at least two elements of the renal SLEDAI in the absence of a biopsy) and 79 patients with non-active renal involvement. Urine samples from patients with active renal involvement had higher numbers of CD4 (median of 1415 cells/100 ml urine), in SLE patients without active renal involvement only low number of T-cells was detected with a median of 29 CD4 T-cells/100ml urine.

Moreover, the present results indicated a statistically significant difference between groups

according to WBC, hemoglobin, 24hr urine protein, serum creatinine, B urea, ESR and CRP. Also, there was a significant difference between the groups according ds-DNA.and C3 and C4 This conclusion is in agreement with that found by ***Mohammad A. Zakaria et al.*** [(11)].

CONCLUSION

Urinary CD4 T-cells marker has a valuable role in detecting LN in SLE patients and has significant correlation with disease activity index. Significant Positive correlation was found between CD4 T-cell and SLEDAI in before and after treatment. Significant positive correlation was observed with 24hr urine protein, SLEDAI, PGA, while Platelet, ESR and C3 showed significant and negative correlation in post treatment. Monitoring urinary CD4 T-cells may help to identify treatment responders and treatment failure and enable patient-tailored therapy in the future.

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