

## Urinary CD4<sup>+</sup>, CD8<sup>+</sup>, CXCR3<sup>+</sup> T cells and IL10 cells in lupus nephritis patients

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### ABSTRACT

Among the most promising novel biomarkers are molecules involved in the recruitment of cells into the kidney and molecules reflecting renal inflammation (cytokines). Prominent examples are urinary CXCL motif and IL10. The aim of the present work is to measure urinary CD4<sup>+</sup>, CD8<sup>+</sup>, chemokine receptor (CXCR3<sup>+</sup>) and interleukine 10 (IL10) in Lupus nephritis patients by flow cytometry in urine sample from 50 patients with LN and 22 healthy controls and correlate these urinary biomarkers with histological class, activity index and chronicity index. The results showed significant increase in CD4<sup>+</sup>, CD4<sup>+</sup>/CD8<sup>+</sup>, CXCR3<sup>+</sup> and IL10 in urine of LN but significant decrease in CD8<sup>+</sup>. CXCR3<sup>+</sup> is negatively correlated with activity index and chronicity index. © 2015 Trade Science Inc. - INDIA

### KEYWORDS

IL10;  
CXCR3<sup>+</sup>;  
CD4<sup>+</sup>;  
CD8<sup>+</sup>;  
LN.

### INTRODUCTION

Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease with the potential to affect a variety of end organs. Lupus nephritis (LN) is one of the most frequent manifestations of SLE and can be present in 60% of SLE patients<sup>[2]</sup>. LN is associated with significant morbidity and mortality and is the most common secondary glomerulonephritis leading to end-stage renal disease<sup>[3]</sup>. Patients with end-stage renal disease require supportive therapy with dialysis or need to undergo renal transplantation, amounting to a huge burden on our healthcare system. Early diagnosis and prompt treatment of LN is associated with significantly better outcome<sup>[10]</sup>. Serological determination of serum anti-double stranded DNA (anti-dsDNA) antibodies and complement levels can be clinically helpful as indi-

cators of disease activity. However, their correlation with lupus renal disease activity in LN is still controversial<sup>[12]</sup>. As it is essential to closely monitor renal disease in LN patients, a none-invasive, easily obtainable and accurate marker to repeatedly assess kidney disease in lupus would be very useful for more precise management.

Studies of murine models of LN as well as studies in SLE patients have uncovered a number of potential disease biomarkers - including chemokines, chemokines receptors, cytokines and that may correlate well with LN<sup>[16]</sup>.

Interleukin (IL)-10 is pleiotropic in its abilities to stimulate B lymphocyte proliferation, immunoglobulin secretion, inhibit T helper type 1 (Th1) responses, promote Th2 responses and to induce the differentiation of regulatory CD4<sup>+</sup> T cells<sup>[6]</sup>. IL10

have been documented to be increased in SLE patients and seem to correlate with disease activity<sup>[22]</sup>. In a murine model of LN, continuous administration of anti-IL-10 antibody to NZB/W F1 mice significantly delayed the development of lupus<sup>[13]</sup>. Moreover, serum IL10 levels have been shown to be elevated in patients with active LN<sup>[5]</sup>.

CXCR3+ is a chemokines receptor that is highly expressed on effector T cells and has been predicted to play an important role in T cell recruitment and immune response in a number of inflammatory and autoimmune diseases<sup>[19]</sup>. In murine lupus, CXCR3 deficiency significantly reduces renal T cell infiltrates and nephritis after induction of nephrotoxic serum nephritis in mice<sup>[18,19]</sup> and results in a significant amelioration of nephritis with reduced tissue damage and T cell infiltration<sup>[25]</sup>. Hence, CXCR3 blockade was recently suggested as a novel therapeutic option for the treatment of lupus nephritis. The recruitment of CXCR3- positive T cells into the kidney has been detected in human and experimental glomerulonephritis<sup>[23]</sup>.

Additionally, it is widely accepted that autoantibody production is T cell dependent<sup>[11]</sup>. The recruitment of inflammatory cells from the circulation into renal tissue is a typical feature of renal inflammatory diseases<sup>[21]</sup>. The activation of autoreactive T lymphocytes leads to the abnormalities of CD4+ and CD8+ T cells, their numbers and also engenders the autoantibody production characteristic of SLE<sup>[17]</sup>. In particular, infiltrating effector T cells of the Th1 type are supposed to initiate renal tissue damage in immune-mediated diseases, eventually leading to progressive loss of renal function<sup>[26]</sup>. Studies have established an important role of chemokines in the regulation of leukocyte migration in renal inflammatory disease<sup>[20]</sup>.

The aim of the present work is to correlate the expression of urine sample CD4+ and CD8+ cells as well as CXCR3+, IL10 T cells in the urine sample of LN patients and their correlation with histological classes, activity index (AI), and chronicity index (CI).

### Subjects and methods

The present study includes the following groups:

1-Healthy control group consisted of 22 healthy children of both sexes their ages (4-12 years)

2- 50 patients of lupus nephritis (LN) group of

both sexes and their ages (4-15 years) from the outpatient of nephrology department of Mansoura University Children's Hospital (MUCH). They were classified according to the International Society of Nephrology/ Renal Pathology Society (ISN/ RPS) 2003: class I was (6), class II was (10), class III was (12), class IV (20), class V was (0), and class VI was (2)<sup>[27]</sup>. Disease activity index (AI) and chronicity index (CI) were assessed by<sup>[1]</sup>. Mean disease activity for all patients was  $7.33 \pm 6.02$ . All patients were treated with a hostacortine (2mg/day), and ste-

**TABLE 1 : Demographic and clinical characteristic of healthy control and lupus nephritis patients.**

Characteristic	Healthy control	LN patients
Total cases	22	50
Age (mean± SD years)	9.68±2.82	9.37±2.65
Sex		
Male (n)	10	10(20%)
Female (n)	12	40 (80%)
Class I	-	5 (10%)
Class II	-	2 (4%)
Class III	-	15 (30%)
Class IV	-	24 (48%)
Class VI	-	4 (8%)

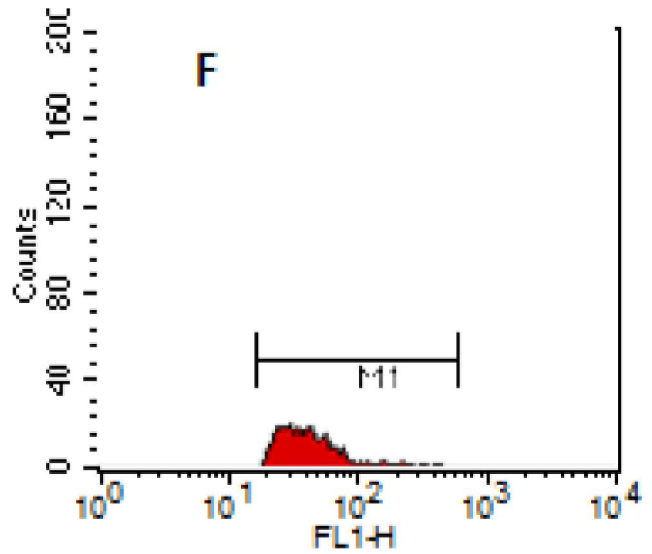
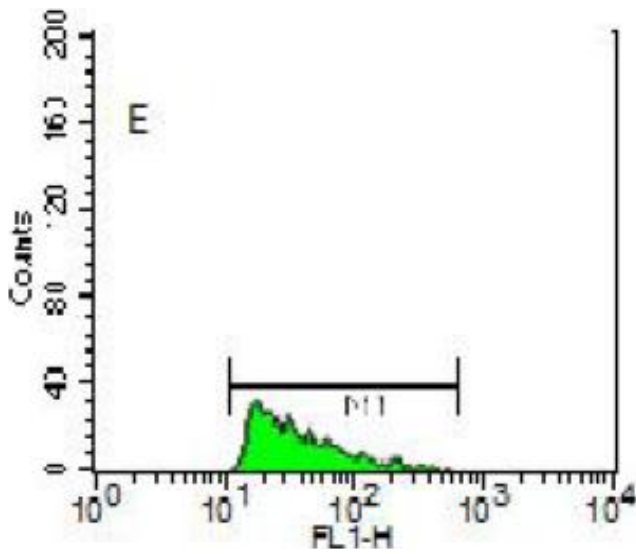
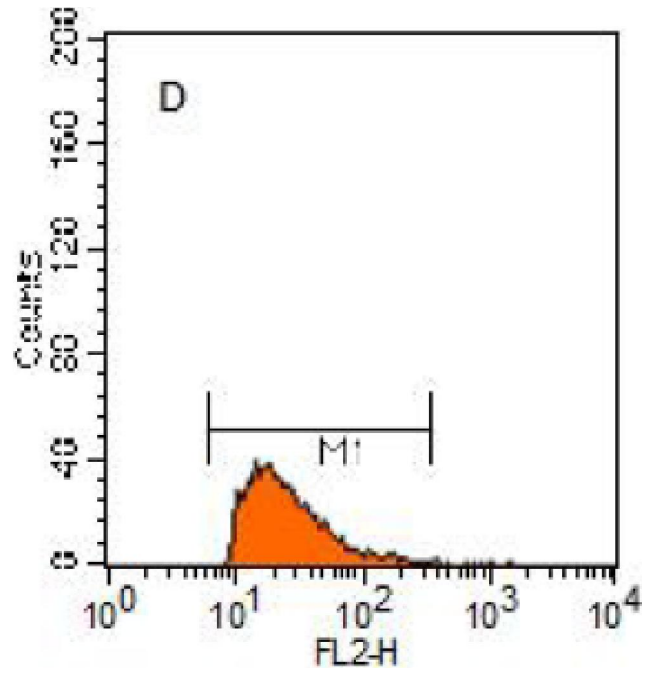
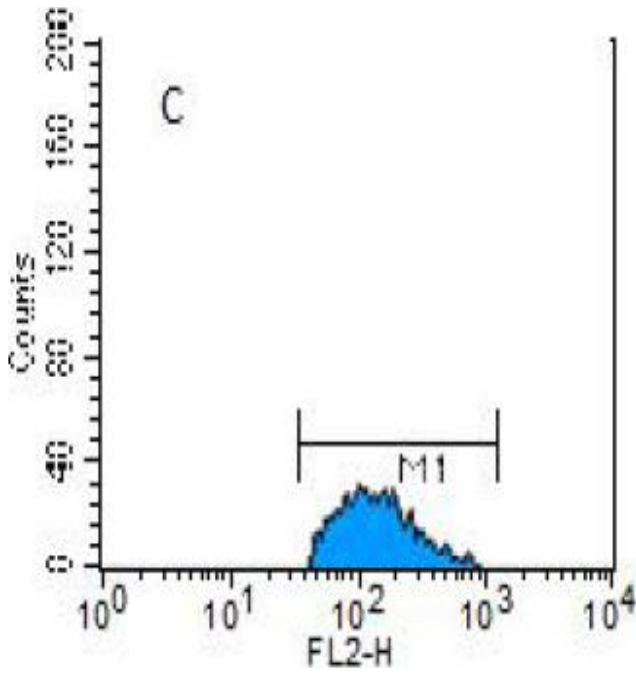
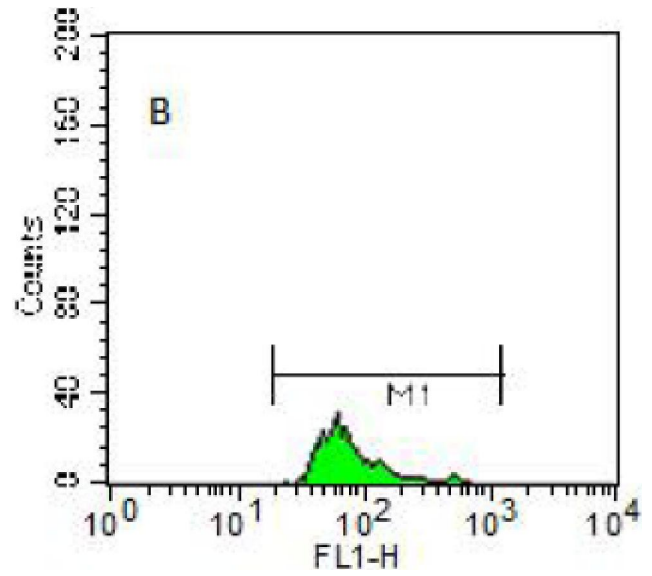
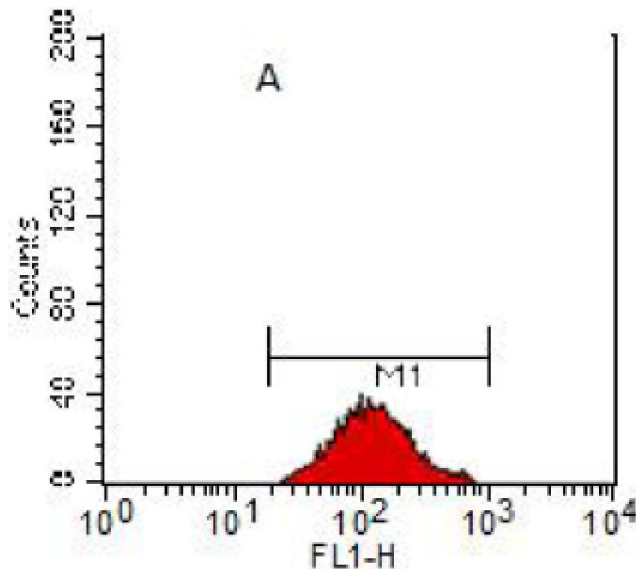
**TABLE 2 : Serum creatinine, urinary protein/creatinine ratio, C3 and ds DNA in control and in LN patients**

	Healthy controls	LN	P
Serum			
Creatinine (Cr) (mg/dl)	0.26±0.07	0.87±0.17	0.001
Urinary			
Protein/Cr ratio	0.04±0.01	0.59±1.06	0.001
C3 (mg/dl)	1.22±0.23	0.92±0.75	0.018
ds- DNA (IU/ml)	7.18±5.84	161.63±111.46	0.001

**TABLE 3 : The frequency of surface markers of estimated parameters (%) in the urine sample of healthy control and Lupus nephritis (LN) patients**

	Healthy controls	LN	P
CD4+	14.96±6.93	30.60±13.52	0.001
CD8+	31.08±6.62	20.15±11.02	0.001
CD4+/CD8+	0.49±0.20	1.68±0.76	0.001
CXCR3+	14.30±5.33	41.31±15.39	0.001
IL10	13.46±3.83	34.66±9.29	0.001
CD4+ CXCR3+	11.42±1.79	2.91±1.98	0.001

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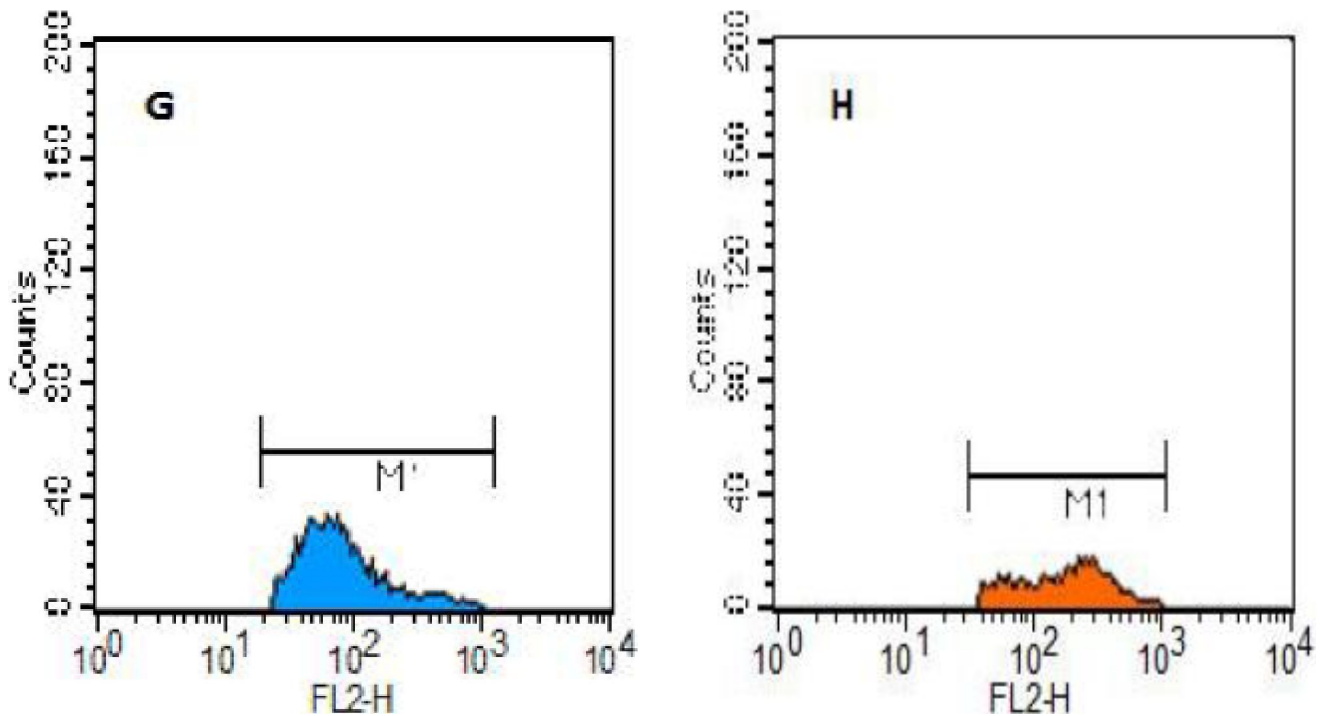


Figure 1 : Flow-cytometry histograms representing the frequency of A: CD4<sup>+</sup> T cells, B: CD8<sup>+</sup> T cells, C: IL10, and D: CXCR3<sup>+</sup> cells from peripheral blood in lupus nephritis (LN) patients and E: CD4<sup>+</sup> T cells, F: CD8<sup>+</sup> T cells, G: IL10, and H: CXCR3<sup>+</sup> cells from healthy control (M1= +ve population)

TABLE 4 : Correlation of estimated parameters with histological class, activity index (AI), and chronicity index (CI) in patients with lupus nephritis (LN)

Parameters	Histological Class	AI	CI
CD4 <sup>+</sup>	r= -.0249 p=NS	r= - 0.244 p=NS	r= 0.026 p=NS
CD8 <sup>+</sup>	r= -.133 p=NS	r= - 0.229 p=NS	r= 0.239 p=NS
CD4 <sup>+</sup> /CD8 <sup>+</sup>	r= 0.340 p=0.016	r=0.243 NS	r= - 0.279 p=0.050
CD4 <sup>+</sup> CXCR3 <sup>+</sup>	r= -.0222 p=NS	r= 0.278 p=NS	r=- 0.060 p=NS
IL10	r= -.066 p=NS	r= - 0.191 p=NS	r= 0.193 p=NS
CXCR3 <sup>+</sup>	r= -.0232 p=NS	r= - 0.362 p=0.010	r= - 0.390 p=0.005

roids (2mg/day).

Urine samples were collected from healthy control and patients. Frequencies of CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells, IL10, and CXCR3<sup>+</sup> cells were determined immediately after sampling by flow cytometry<sup>[7]</sup>. Serum creatinine, serum C3, serum ds-DNA and uri-

nary protein were determined.

The following antibodies were used in flow cytometry: fluorescein (FITC)-conjugated anti-CD4 (clone OKT4), Phycoerytherine (PE) - conjugated anti-CXCR3 (clone CXCR3- 183) and Phycoerytherine (PE)- conjugated anti- IL10 (clone JES3-9D7), All were purchased from Biologend, Sandi ago, USA).

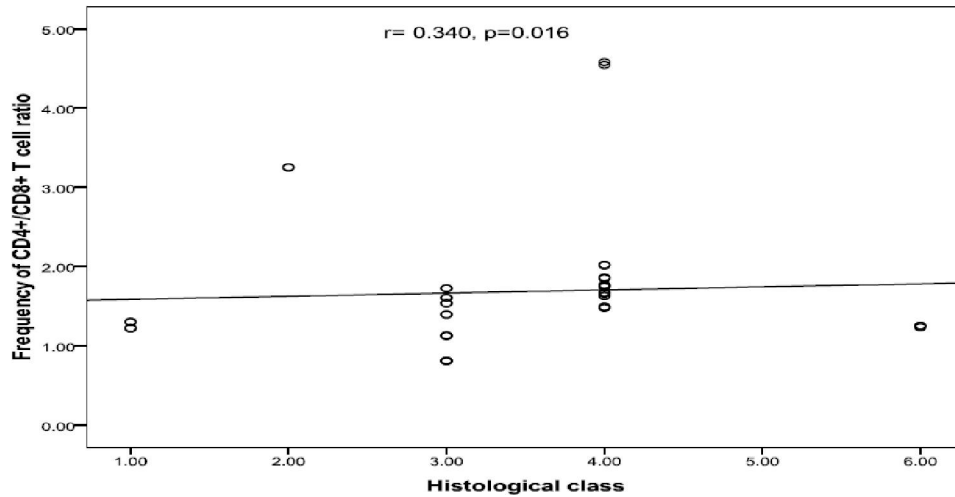
**Statistical analysis**

Data were analyzed using SPSS 19.0 for windows (SPSS Inc., Chicago, IL, USA). The tested parameters were compared using t-test student. The correlation coefficient was obtained by the parametric Pearson correlation test. P values less than 0.05 were considered to be significant. The non-parametric parameters were compared using Mann-Whitney U test. The correlation coefficient of non-parametric parameters was obtained by Spearman’s correlation test<sup>[14]</sup>.

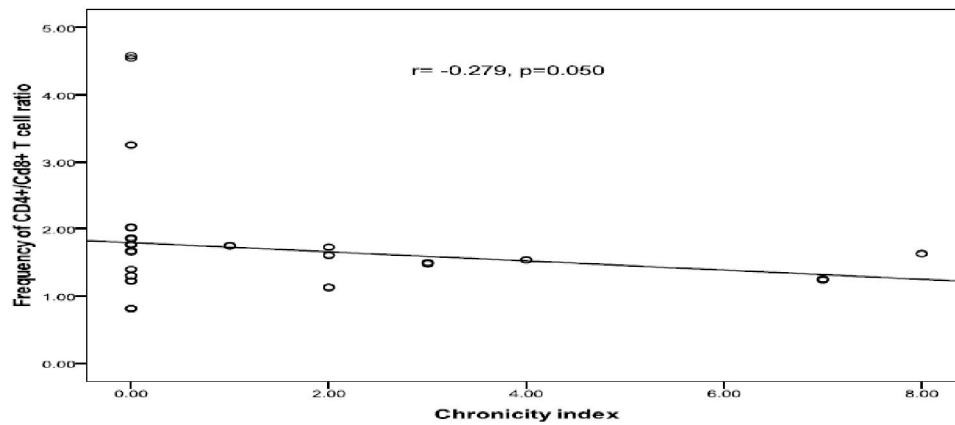
**RESULTS**

TABLE 2 showed that patients with LN have significant (p=0.001) increase in serum creatinine, urinary protein/creatinine ratio, and anti-dsDNA, but significant decrease (0.018) in complement C3

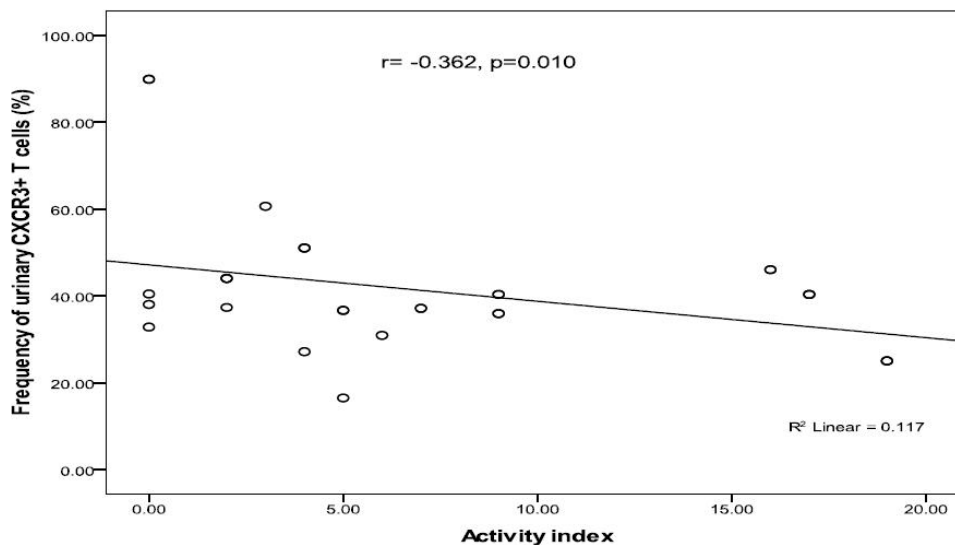
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**Figure 2 :** Correlation between the urinary CD4<sup>+</sup>/CD8<sup>+</sup> T cells ratio and histological class of lupus nephritis patients.



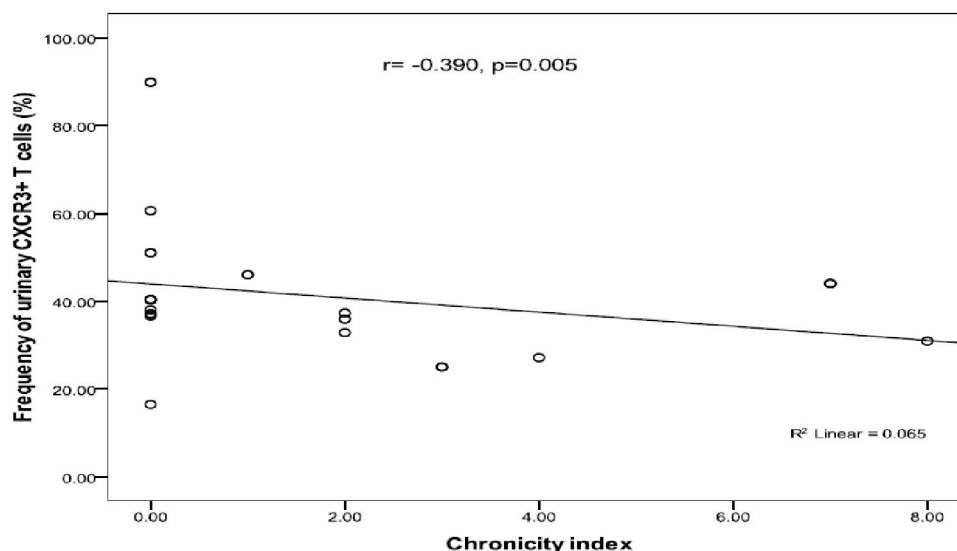
**Figure 3 :** Correlation between the urinary of CD4<sup>+</sup>/CD8<sup>+</sup> T cells ratio and chronicity index of lupus nephritis patients.



**Figure 4 :** Correlation between the frequency urinary CXCR3<sup>+</sup> cells (%) and activity index of lupus nephritis patients.

As shown in TABLE 3 and Figure 1 the frequency of urine sample CD4<sup>+</sup> T cells (%) of LN patients was significantly increased as compared to healthy control group (30.60±13.52 vs 14.96±6.93, p=0.001). While, the frequency of urinary CD8<sup>+</sup> T cells (%) obtained

from LN patient was significantly decreased as compared to healthy control (20.15±11.02 vs 31.08±6.62, p= 0.001). Also, there is a significant increase in the frequency of CD4<sup>+</sup>/CD8<sup>+</sup> T cells ratio (%) of LN patient as compared to healthy control (1.68±0.76 vs



**Figure 5 :** Correlation between the frequency urinary CXCR3<sup>+</sup> cells (%) and chronicity index of lupus nephritis patients.

0.49±0.20, p=0.001). Also, there is a significant increase in the frequency of IL10 (%) in LN patients as compared to healthy control (34.66±9.29 vs 13.46±3.83, p= 0.001). In addition, there is significant decrease in the frequency of urinary CD4<sup>+</sup>CXCR3<sup>+</sup> cells (%) as compared to healthy control (2.91±1.98 vs 11.42±1.79, p=0.001).

The obtained data from TABLE 4 and Figure 2-5 showed that the frequency of urinary CXCR3<sup>+</sup> cells (%) was negatively correlated with AI (r= -0.362, p=0.010). While, the frequency of CD4<sup>+</sup>/CD8<sup>+</sup> T cells (%) ratio was positively correlated with histological class, and CI (r=0.340, p=0.016, r= -0.279, p= 0.050, respectively). Furthermore, the frequency of urinary CXCR3<sup>+</sup> (%) was negatively correlated with AI (r= -0.362, p=0.010).

## DISCUSSION

Systemic lupus erythematosus is a chronic inflammatory with unknown etiology. About 60% of SLE patients develop LN during the course of their disease<sup>[2]</sup>. Abnormality in T cell function is considered as one of the universal factors in the pathogenesis of SLE<sup>[24]</sup>.

As seen in the present work, there was significant increase in the urinary CD4<sup>+</sup> T cells, this is agreement with<sup>[9]</sup> who concluded that urinary CD4<sup>+</sup> T cells are highly sensitive and specific markers for detecting proliferative LN in patients with SLE.

Dolff et al.<sup>[8]</sup> Found increase urinary CD8<sup>+</sup> T cells, however the present study urinary CD8<sup>+</sup> was significant decrease<sup>[8]</sup>.

The observed increase in urinary CXCR3<sup>+</sup> in LN patients. This result was also reported by Avihingsanon et al., (2006) who found correlation with disease activity being upregulated in patients with active LN and not detectable in healthy control. However, in the present study CXCR3<sup>+</sup> is inversely correlated with both activity index and chronicity index.

Increase urinary IL10 in this study is agreement with<sup>[15]</sup>. However<sup>[4]</sup> found decreased urinary IL10.

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