Anti-C1q Antibodies, sCD40L, TWEAK and CD4/CD8 Ratio in Systemic Lupus Erythematosus and Their Relations to Disease Activity and Renal Involvement

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Due to the unpredictable nature of lupus nephritis (LN), it would be clinically valuable to discover a reliable biomarker for disease activity and progression. The aim of this study is to evaluate the role of anti-C1q antibodies, sCD40L and TWEAK in systemic lupus erythematosus (SLE) and their relation to disease activity and kidney involvement. This study included 47 patients with SLE, 28 with LN and 19 without LN, as well as, 20 healthy subjects as controls. All subjects underwent complete history, examination and estimation of disease activity index (SLEDAI) and renal SLEDAI. The following investigations were done for all subjects: anti-C1q antibodies, sCD40L, TWEAK and CD4/CD8 ratio, in addition to complete blood picture, ESR, kidney function tests, ANA, anti-ds DNA antibodies and C3, C4. Anti-C1q antibodies, sCD40L and TWEAK and anti-dsDNA were significantly higher in SLE patients than controls (*P*<0.001 for each), while C3, C4 and CD4/CD8 ratio were significantly lower (*P*<0.001, 0.05 and 0.001 respectively). In LN patients, anti-C1q antibodies, sCD40L and TWEAK were significantly higher than non LN patients (*P*<0.001 for each). Anti-C1q antibodies, sCD40L and TWEAK correlated with traditional disease activity parameters (C3, C4, anti-dsDNA and SLEDAI) as well as rSLEDAI. Levels of serum TWEAK correlated with the development of LN in patients with SLE. We concluded that anti-C1q antibodies, sCD40L and TWEAK may be used as serum biomarkers for the assessment of disease activity and development of LN.

Lupus nephritis (LN) remains a major cause of morbidity and mortality in systemic lupus erythematosus (SLE) patients. Overt renal disease is found in at least 30 to 50% of SLE patients (Cross & Jayne, 2005). Early diagnosis and prompt treatment may significantly improve long-term prognosis. Serological determination of serum anti-double stranded DNA (anti-dsDNA) antibodies and complement levels can be clinically helpful as indicator of disease activity (Schwartz *et al.,* 2006). However, the correlation between these markers and lupus renal disease is imperfect and their usefulness in reflecting disease activity and in predicting outcome in LN is still controversial (Illei *et al.,* 2004). It is essential to closely monitor renal disease in LN patients; especially since disease activity may fluctuate thus these patients would require treatment modification. A non invasive, accurate and easily obtainable biomarker will give clinicians a dependable mean to repeatedly assess kidney disease in lupus, and perhaps aid in predicting LN progression (Schwartz *et al.,* 2007).

The complement system plays a major role in innate immunity. Activation of the classical pathway initiated by immune complexes lead to lysis of target structures. In SLE, many abnormalities in the activation of the complement system have been reported (Horák *et al.,* 2006). The role of C1q in clearance of immune complexes and apoptitc cells is the probable mechanism linking its deficiency or inhibition with the development of SLE (Bowness *et al.,* 1994; Walport, 2001). It has been suggested that the presence of anti-C1q leads to the development of LN (Fremeaux-Bacchi *et al.,* 2002), thereby monitoring anti–C1q levels might be valuable

for the clinical management of SLE patients (Siegert *et al.,* 1999; Marto *et al.,* 2005).

CD40 ligand (L) is a type II membrane protein of 33 kDa which is normally expressed on Th1, Th2 cells and activated platelets (Otterdal *et al.,* 2004). CD40L circulate in a soluble form of 18-20 kDa in the serum of patients with SLE (Vakkalanka *et al.,* 1999). The soluble CD40L has been associated with disease activity and severity (Kato *et al.,* 1999). The involvement of CD40-CD40L pathway in the pathogenesis of LN has been documented by several studies in humans (Nagafuchi *et al.,* 2003), and in murine models (Higuchi *et al.,* 2002). In addition the blockade of the pathway by anti-CD40L antibodies has ameliorated the clinical and laboratory parameters of nephritis in both mice (Quezada *et al.,* 2003) and humans (Boumpas *et al.,* 2003).

TNF – like weak inducer of apoptosis (TWEAK), a TNF–ligand superfamily member, is synthesized as a 249 amino acid type II transmembrane protein (Chicheportiche *et al.,* 1997). Fibroblast growth factor-inducible 14 (Fn14) has been identified to be a TWEAK receptor (Wiley *et al.,* 2001). Similar to TNF, TWEAK is processed into a soluble form that circulates as a trimer; it is believed to be the primary mediator of its biological effects (Schwartz *et al.,* 2006). TWEAK is mainly produced by monocytes and macrophages, and is widely expressed at the mRNA level in tissues, including kidneys' tissues (Jakubowski *et al.,* 2002; Wiley & Winkles, 2003). TWEAK regulates cell proliferation, cell death and inflammation; it induces mesangial cells, podocytes and endothelial cells to secrete chemokines (Schwartz *et al.,* 2006). Increased TWEAK and Fn receptor expression in tubular cells was reported during acute kidney injury, as well as the presence of elevated urinary TWEAK levels in patients with active LN. TWEAK may be a new player in kidney injury and might be a target for therapeutic intervention (Sanz *et al.,* 2008).

The pathogenic importance of T cells in SLE is widely recognized (Horowitz *et al.,* 1997). Although the cause of SLE is unknown, several investigations have revealed immunoregulatory abnormalities that may underlie the autoimmune reactivity. Multiple defects of the functions of T and B cells functions are found in patients with SLE. The numbers of T lymphocytes are decreased and T cell functions are impaired (Keskin *et al.,* 1996). Previous studies have found either a normal or an increase level of $CD8^+$ T cells and a decrease in $CD4^+$ T cells, with a consequent decrease of the CD4/CD8 ratio, in patients with active SLE, especially those with severe LN (Bakke *et al.,* 1983).

The aim of this study is to measure serum levels of anti-C1q antibodies, sCD40L and TWEAK and CD4/CD8 ratio in peripheral blood of SLE patients and correlate these parameters with disease activity and the development of LN.

Subjects and Methods

Subjects

Forty-seven Egyptian females with SLE were admitted to the Department of Internal Medicine, Rheumatology Unit, Assiut University Hospitals from September 2007- September 2008. Full history and clinical examination were performed for all the participants. The diagnosis of SLE was made following the criteria set by The American College of Rheumatology (Hochberg, 1997). Their mean age was 25.26±7.22 and the mean value of the disease duration was 2.57±3.1 years. The medication was stopped one week before the study. Of these, 28 patients had LN as confirmed by renal biopsy. Twenty healthy females with comparable age were also included as controls. Informed consents were obtained from all the participants. The experimental design was approved by the Ethics Committee (Faculty of Medicine).

Assessment of Disease Activity: Systemic lupus erythematosus disease activity index (SLEDAI) was used to evaluate disease activity (Bombardier *et al.,* 1992). Kidney disease activity was assessed by renal SLEDAI (rSLEDAI) score that consists of the 4 kidney-related items of the SLEDAI 2000 (SLEDAI-

2k), (hematuria, pyuria, proteinuria, and urinary cast) (Gladman *et al.*, 2002). The presence of each one of the 4 parameters gives a score of 4 points; thus, rSLEDAI score can range from 0 (non-active renal disease) to a maximum score of 16. The LN patients divided into low kidney disease activity with score = 4 and high kidney disease activity with score ≥ 8.

Laboratory investigations were performed for every subject. 10 ml of venous blood were aseptically collected, 3 ml in tubes containing lithium heparin as anticoagulant, for Flowcytometric analysis, 1 ml in tubes containing EDTA for complete blood picture, and 1.6 ml in tubes containing 3.8% sodium citrate for ESR. The serum of remaining was aliquoted and stored at -20°C for biochemical tests.

Complete blood count (CBC) was performed using Beckman Coulter HMX. ESR was done according to Westergren.

Kidney function tests, urea, creatinine and uric acid were performed using a chemical analyzer Hitachi 911 (Boehringer Mannheim, Germany). Complete urine analysis using reagent strips 10 parameters (polypharma), protein in 24 hours (sulfosalicylic acid method and creatinine clearance (Jaffé method and creatinine clearance is calculated (Rock *et al.*, 1987).

Antinuclear antibody (ANA) titers were determined by the indirect immuno-fluorescence (IF) technique (Dia Sorine, HEP2 cell line substrate). Patient serum samples diluted in buffer were overlaid onto HEp-2 cells grown on a microscope slide. The resultant antigen-antibody complexes bound fluorescein labeled antihuman immunoglobulin (apple-green fluorescence) was visualized using fluorescence microscope (NS200, Nachet, Levallois, France).

C3 and C4: Serum levels of C3 and C4 was assessed using the single radial immunodiffusion technique (The Binding site Ltd, Birmingham, UK). Three calibrators were included to produce a linear calibration curve. After 48 hours incubation period, ring diameters formed were measured. The normal range for C3 and C4 in female is 1032-1495 mg/L and 167-385 mg/L, respectively, as mentioned in test manual.

Anti-dsDNA: The BINDAZYME^{TM} Human Anti-dsDNA Enzyme Immunoassay is indented for in-vitro measurement of specific IgG autoantibodies against double stranded deoxyribonucleic acid present in human serum (Cat. No. MK017; The Binding Site Ltd, Birmingham, UK). Microwells pre-coated with calf thymus dsDNA antigen were used. The calibrators, controls and diluted patient samples were added to the wells, for 30 min at room temperature. After washing the wells to remove all unbound proteins, purified peroxidase labeled rabbit anti-human IgG conjugate was added and the excess unbound conjugate was removed by washing. The bound conjugate is visualized with 3,3',5,5' tetramethylbenzidine(TMB) substrate which gives a blue reaction product which intensity is proportional to the concentration of autoantibody present in the sample. Phosphoric acid was added to stop the reaction and the produced yellow end point colour was read at 450 nm (Stat Fax-2100, Awareness Technology Inc., USA)

Anti-C1q autoantibodies: The BINDAZYME^{TM} Human Anti-C1q Enzyme Immunoassay for the determination of serum anti-C1q IgG autoantibodies; Cat. No MK079 (Binding SiteLtd, Birmingham, UK). Microwells are pre-coated with purified human C1q. The calibrators, controls and diluted patient samples were added to the wells, for 30min at room temperature. After washing the wells, purified peroxidase labelled rabbit anti-human IgG (γ chain specific) was added to all wells. Following washing, the reaction was visualized using 3,3,5,5. tetramethylbenzidine (TMB) substrate. Phosphoric acid is added to each well to stop the reaction and absorbance of the developed colour was measured at 450 nm (Stat Fax-2100, Awareness Technology Inc, USA).

sCD40L

The determination of soluble CD40L in serum was performed using ELISA (Cat. No; KHS4001; BIOSOURCE, USA) according to manufacturer's instructions. Briefly, patient' sera was allowed to react with anti-sCD40L monoclonal antibody adsorbed onto microwells. HRP-conjugated monoclonal anti-sCD40L antibody was added to wells, 2 hours at room temperature, on a rotator set at 100 rpm. Following washing, substrate solution was added. After stopping reaction with acid, absorbance of the developed colored reactions was measured at 450 nm (Stat Fax-2100, Awareness Technology Inc, USA).

TWEAK

Human TWEAK ELISA was used for the quantitative detection of human TWEAK in serum according to manufacturer's instructions (Bender MedSystems, USA). Briefly, an anti-human TWEAK polyclonal antibody adsorbed onto microwells was used to capture Human TWEAK in patient's serum. Addition of biotin-conjugated polyclonal anti-human TWEAK antibody was followed by addition of Streptavidin-HRP. Following washing, substrate solution was then added. Reaction was stopped with acidic solution and absorbance of the developed colored reaction was

measured at 450 nm (Stat Fax-2100, Awareness Technology Inc, USA).

CD4 $^{+}$and CD8^{+} T cells were assessed in patient's peripheral blood using flowcytometry. Dual color monoclonal antibodies were used to identify the lymphocyte subsets in the peripheral blood of both SLE patients and controls. CD45 for lymphocyte gating, CD3/CD19 for identifying T and B cells, CD4 FITC /CD8 PE for identifying T cell sub sets. The mononuclear cells were isolated on a high density gradient separation medium (Ficoll Hypaque). The cells were washed twice in phosphate buffered saline (PBS, PH 7.4+0.2) at 400 x g for 4 min. Cells were then incubated with FITC conjugated anti-CD4 mAb and PE conjugated anti –CD8 mAb (Dako MultiMix^{TM}) for 30 min at 4^{o}C. Following incubation time, the cells were washed twice in PBS with 2% albumin at 400 x g for 4 min and then suspended into 300 µl PBS. The purity of T lymphocyte was assessed by flowcytometry (PAS-II Dako –Cytomation). FlowMax software was used for acquisition and analysis.

Statistical Analysis

All data were expressed as mean ± SD and percentages. Comparisons between two groups were analyzed by Student t-test and Chi square. Correlation between studied parameters was performed by Spearman rank correlation coefficient. *P* value < 0.05 was considered significant. SPSS statistical software (version 11) was used for statistical calculation.

Results

Demographic and clinical data obtained for studied groups, SLE with or without renal involvement were similar for both groups (Table 1).

Table 1: Demographic Data of Studied Patients.

Parameters	All SLE patients n=47	SLE patients with LN n=28	SLE patients without LN n=19	*P* value
Age (years)	25.26±7.22	25.75±6.26	24.53±8.57	NS
Duration(years)	2.57±3.1	2.46±2.97	2.73±3.34	NS
Arthritis/arthralgia	45 (95.7%)	26 (92.9%)	19 (100%)	NS
Malar rash	36 (76.6%)	18 (64.3%)	18 (94.7%)	<0.05
Photosensitivity	26 (55.3%)	18 (64.3%)	8 (42.1%)	NS
Alopecia	46 (97.9%)	27 (96.4%)	19 (100%)	NS
Cardiac involvement	4 (8.5%)	3 (10.7%)	1 (5.3%)	NS
Pulmonary disorders	8 (17%)	4 (14.3%)	4 (21.1%)	NS
Vasculitis	7 (14.9%)	4 (14.3%)	3 (15.8%)	NS
Central Nervous System involvement	2 (4.3%)	1 (3.6 %)	1 (5.3%)	NS
Hypertension	8 (17%)	7 (25%)	1 (5.3%)	NS
Lupus Nephritis	28 (59.57%)	28 (100%)	---	---
ESR mm/h	74.42±39.16	72.67±42.41	76.78±35.14	NS
Hemoglobin gm/L	8.71±2.19	8.15±1.81	9.44±2.45	NS
WBCs × 10^9/L	6.55±4.05	7.13±4.10	5.77±3.96	NS
Platelets × 10^9/L	242.37±132.21	204.95±128.85	288.59±124.75	NS
ANA	45 (95.7%)	27 (96.4%)	18 (94.7%)	NS
SLEDAI	16.64±5.73	20.11±4.88	11.53±1.43	<0.001
rSLEDAI	4.17±4.49	7.00 ±3.71	0	----

NS= not significant

The levels of anti-dsDNA, anti-C1q, sCD40L and TWEAK were significantly higher (P<0.001) in SLE patients than in controls. On the other hand, the levels of C3, C4, CD4/CD8 ratio were significantly lower (*P*<0.001, 0.05, and 0.001, respectively) in SLE patients than controls (Table 2).

The mean values of anti-C1q, sCD40L and TWEAK were significantly higher (*P*< 0.001) in LN patients than non LN patients (Table 3).

Table 2. Serum Levels of Studied Parameters in SLE Patients and Controls

Parameters (mean±SD/ range)	SLE(47)	Controls(20)	*P* value
C3(mg/L)	799.15±497.76 172.00-1890.00	1372.00±269.10 1040.00-1890.00	<0.001
C4 (mg/L)	189.10±154.37 58.00-773.10	283.40±138.05 140.00-580.00	<0.05
Anti-dsDNA (IU/ml)	535.10±589.68 5.00-1780.00	16.00±3.56 12.30-25.00	<0.001
Anti-C1q (U/ml)	32.61±39.90 2.70-130.00	2.27±1.00 0.12-4.10	<0.001
sCD40L ng/ml	4.97±4.35 0.50-26.00	1.30±0.61 0.50-2.00	<0.001
TWEAK(pg/ml)	678.72±540.91 60.00-1800.00	104.30±27.53 60.00-144.00	<0.001
CD4%	34.77±2.91 30.00-40.00	45.30±4.03 40.00-55.00	<0.001
CD8%	30.02±1.89 26.00-35.00	24.40±1.79 22.00-29.00	<0.001
CD4/CD8 ratio	1.13±0.10 0.90-1.30	1.83±0.06 1.70-1.90	<0.001

Table 3. Serum Levels of Studied Parameters in LN and Non LN Patients.

Parameters (mean±SD)	LN(28)	Non LN(19)	*P* value
C3(mg/L)	693.14±445.5	955.37±540.58	NS
C4 (mg/L)	152.18±450.58	243.50±148.19	NS
Anti-ds DNA (IU/ml)	595.81±627.16	445.63±533.35	NS
Anti-C1q (U/ml)	47.92±44.77	10.06. ±12.71	<0.001
sCD40L (ng/ml)	6.93±4.65	2.09±1.11	<0.001
TWEAK (pg/ml)	1044.79±391.76	139.20±43.24	<0.001
CD4%	34.61±3.14	35.00±2.60	NS
CD8%	30.20±1.891	29.73±1.91	NS
CD4/CD8 ratio	1.11±0.12	1.14±0.018	NS

NS= not significant

Only, the level of anti-C1q was significantly higher (P<0.01) in LN group with high disease activity when compared to low active group, while the other parameters showed no significant difference (Table 4).

Table 4. Serum Levels of Studied Parameters in High Active and Low Active LN Patients.

Parameters (mean±SD)	High activity(14)	Low activity(14)	*P* value
C3(mg/L)	592.00±292.422	794.29±551.96	NS
C4 (mg/L)	121.66±81.34	182.69±194.00	NS
Anti-dsDNA(IU/ml)	755.76±686.52	435.86±538.98	NS
Anti-C1q (U/ml)	70.33±50.26	25.51±23.54	<0.01
sCD40L ng/ml	7.75±5.96	6.11±2.80	NS
TWEAK (pg/ml)	1094.57±377.62	995.0±413.30	NS
CD4/CD8 ratio	1.08±0.11	1.15±0.11	NS

NS= not significant

A significant (P<0.001) positive correlation was observed between anti-C1q antibodies with SLEDAI, rSLEDAI, anti-dsDNA, sCD40L and TWEAK and a significant (P<0.001, 0.01 and 0.001) negative correlation with C3, C4 and CD4/CD8 ratio, respectively. A positive correlation was found between sCD40L with SLEDAI, rSLEDAI, anti-dsDNA and TWEAK (<0.001 for all) and a negative correlation with C3, C4 and

CD4/CD8 ratio (*P*<0.01, 0.01 and 0.001, respectively). With respect to serum TWEAK, it correlated positively (*P*<0.001) with SLEDAI and rSLEDAI and correlated negatively (*P*<0.001, 0.01, 0.001) with C3, C4 and CD4/CD8, respectively (Table 5).

Table 5. Correlation between Studied Variables

Parameters	Anti- C1q	sCD40L	TWEAK	CD4/CD8 ratio
C3 (mg/L)	r=-0.423 *P*<0.001	r=-0.349 *P*<0.01	r=-0.449 *P*<0.001	r=0.365 *P*<0.01
C4(mg/L)	r=-0.364 *P*<0.01	r=-0.349 *P*<0.01	r=-0.311 *P*<0.01	r=0.366 *P*<0.01
Anti-dsDNA (IU/ml)	r=0.699 *P*<0.001	r=0.428 *P*<0.001	r=0.455 *P*<0.001	r=-0.644 *P*<0.001
Urea (mmol/L)	r =0.471 *P*<0.001	r =0.482 *P*<0.01	r =0.443 *P*<0.01	NS
Creatinine (µmol/L)	r=0.407 *P*<0.001	r =0.345 *P*<0.05	NS	NS
Creatinine Clearance	NS	NS	NS	NS
Proteinuria (g/day)	r =0.431 *P*<0.001	r=0.756 *P*<0.001	r =758 *P*<0.001	NS
SLEDAI	r=0.511 *P*<0.001	r=0.606 *P*<0.001	r=0.588 *P*<0.001	r=-0.344 *P*<0.05
rSLEDAI	r=0.606 *P*<0.001	r=0.690 *P*<0.001	r=0.764 *P*<0.001	r=-0.296 *P*<0.05
Anti-C1q (U/ml)		r=0.509 *P*<0.001	r=0.697 *P*<0.001	r=-0.632 *P*<0.001
sCD40L(ng/ml)	r=0.594 *P*<0.001		r=0.726 *P*<0.001	r=-0.576 *P*<0.001
TWEAK (pg/ml)	r =0.697 *P*<0.001	r=0.735 *P*<0.001		r==-0.577 *P*<0.001
CD4 %	r= -0.631 *P*<0.001	r=-0.521 *P*<0.001	r==-0.486 *P*<0.001	r=0.863 *P*<0.001
CD8 %	r=-0.582 *P*<0.001	r=0.493 *P*<0.001	r=0.549 *P*<0.001	r=-0.771 *P*<0.001
CD4/CD8 ratio	r=-0.660 *P*<0.001	r=-0.576 *P*<0.001	r =-0.565 *P*<0.001	

NS= not significant

Discussion

Systemic lupus erythematosus is a complex and complicated autoimmune disease characterized by clinical involvement of all organs (especially kidneys). Many immunological abnormalities were reported in association with SLE pathogenesis such as the extensive production of autoantibodies, impaired apoptosis, and complement deficiencies or consumption (Horák *et al.*, 2006; Ciferská *et al.*, 2007). Most importantly, many studies about LN support that the timing of treatment is important in prognostic significance, and treatment delay is linked to worse outcomes (Korbet *et al.*, 2000). This study was designed to clarify the role of anti-C1q, sCD40L and TWEAK testing in the early detection and monitoring of LN development among SLE patients.

The present study showed that higher levels of anti-C1q antibodies were detected in SLE patients than in controls; moreover LN patients had also higher levels of anti-C1q antibodies than in SLE patients without LN. Our results corroborate previous studies reporting a strong association between anti-C1q and LN. (Horák *et al.*, 2006, Marto *et al.*, 2005, Mosca *et al.*, 2006; Moura *et al.*, 2009). Earlier investigations suggested that development or recurrence of nephritis was associated with rising titers of anti- C1q during active renal involvement and they were more specific than anti-dsDNA in detecting disease (Coremens *et al.*, 1995; Horvath *et al.*, 2001; Moroni *et al.*, 2001). Comparing patients with low and high activity of LN, defined according to SLEDAI score, higher levels of anti-C1q antibodies were found in patients with highly active LN. The absence of anti-C1q antibodies in serum excludes a diagnosis of LN, whereas increased levels may predict renal flares (Moroni *et al.*, 2001; Lee & Madaio, 2008).

It is still intriguing how antibodies to C1q could cause renal disease in SLE. Due to defective uptake and clearance of apoptotic cells, the exposure of nucleosomes to the immune system results in the production of autoantibodies against several lupus antigens, such as dsDNA and nucleosomes. Circulating immune complex deposit in the glomerulus and these are recognized by C1q. As a result of this binding, the neoantigen within C1q molecule will serve as a focus for circulating anti-C1q autoantibodies. Consequently, full activation of the classical pathway of the complement will result leading to tissue injury mediated by the membrane attack complex as well as the influx of inflammatory cells which exert their effector functions (Trouw & Daha, 2005; Flierman & Daha, 2007). Data obtained in this study showed inverse correlations between anti-C1q antibodies and C3, C4, which were similar to previous results (Horák *et al.*, 2006; Marto *et al.*, 2005; Moura *et al.*, 2009). Also a significant positive correlations were found between anti-C1q antibodies and anti-dsDNA, SLEDAI and rSLEDAI, which was in agreement with the studies of Braun *et al.* (2007); Horák *et al.* (2006) and Ciferská *et al.* (2007).

In this study, the highly significant differences in sCD40L serum levels show the stratification of this parameter in SLE disease. The correlation with C3, C4 and anti-C1q antibodies are important finding from the point of view of interaction of T and B cells proliferation and complement activation. Previous studies showed higher levels of sCD40L in SLE patients than controls (Goules *et al.*, 2006; Ciferská *et al.*, 2007). The positive correlations between sCD40L with SLEDAI and anti-dsDNA, confirm the previous studies that correlate the presence of sCD40L with disease activity (Kato *et al.*, 1999). sCD40L is considered as a proinflammatory and prothrombotic cytokine (Aggarwal *et al.*, 2004). In contrast, Ciferská *et al.* (2007) did not find any correlation between sCD40L and disease activity.

Beside antibodies formation, the impaired interaction between T and B cells are another example of modification of immune response in SLE (Hahn *et al.,* 2005). CD40 is a transmembrane protein expressed on surface of antigen-presenting cells including B cells, activated macrophage, dendritic cells and monocytes (Hill & Lunec, 1996). The corresponding ligand is CD40L, which is found on the surface of T helper cells. CD40-CD40L ligation provides the signals required for B-cell activation and differentiation, immunoglobulin class switching, and up-and down regulation of their apoptosis (Goules *et al.,* 2006). The abnormal expression of key signaling molecules and the defective signal transduction pathways between T and B cells are behind the survival of autoimmune B clones and overproduction of antibodies (Hahn *et al.,* 2005; Ciferská *et al.,* 2007).

As regards the role of sCD40L in development of LN, this study showed significantly higher levels of sCD40L in LN patients than SLE patients without LN. Also, sCD40L correlated positively with rSLEDAI, however its levels tend to be high in highly active disease than low active disease but did not reach statistically significant levels. In experimental murine models, the effectiveness of anti-CD40L therapy in LN was demonstrated (Kalled *et al.,* 1998; Quezada *et al.,* 2003). In the initial phase of LN, auto-antibody dependent reactions occurs first. As LN disease progresses, cell to–cell interactions are needed including the activation of CD40-CD40L pathway to maintain the tissue injury (Kuroiwa *et al.,* 2000).

Cognate interactions between members of the TNF –ligand superfamily and TNF –receptor superfamily members are pivotal in the maintenance of normal immune homeostasis, and are also involved in the pathogenesis of autoimmune disease. TWEAK, a member of TNF –ligand superfamily, is a type II transmembrane protein that is cleaved to form the biologically active circulating trimeric complex (Chicheportiche *et al.,* 1997).

The present study demonstrated significantly higher levels of TWEAK in SLE patients than controls and in LN patients than non LN patients. To our knowledge, no such work was done before, but Schwartz *et al.* (2006), found that patients with active LN had significantly higher urinary TWEAK as compared to individual with inactive LN or those who never had renal involvement. Furthermore, urinary TWEAK correlated with C3, C4, SLEDAI and rSLEDAI. Other studies showed higher levels of serum TWEAK in rheumatoid arthritis and its levels correlated positively with disease activity score (Park *et al.,* 2008).

TWEAK is believed to have a role in the pathogenesis of SLE, but inadequately explored. In the experimental model, TWEAK mRNA is highly expressed in kidneys of male mice early in the disease process. While TWEAK expression is downregulated as mice age and nephritis become manifest (Chicheportiche *et al.,* 2000). Additional support for a possible role of TWEAK in the etiopathogenesis of human SLE is based on the ability of TWEAK to induce macrophage apoptosis (Kaplan *et al.,* 2002). However, the pro-apoptotic effects of TWEAK are relatively weak, and it is not clear if increased apoptosis is indeed a key feature in pathogenesis of human SLE. So it had been believed that the major role of TWEAK in LN is centered on its proinflammatory chemokine inducing effects (Campbell *et al.,* 2004).

Human mesangial cell, podocytes and tubular cells express Fn14, and are TWEAK responsive (Gao *et al.,* 2009). TWEAK stimulated the three types of kidney cells to induce potent proinflammatory response by secretion of chemokines, which are pivotal in the pathogenesis of many inflammatory renal

diseases (Schwartch *et al.,* 2006; Zhoa *et al.*, 2007). Recently Gao *et al.,* (2009) found that TWEAK induce human kidney cells to express multiple inflammatory mediators, including RANTES, MCP-1 IP-10, MIP I α, ICAM and VCAM. Cytokine production is mediated through NF-kB activation, and is inhibited by anti-TWEAK monoclonal antibodies. TWEAK stimulate chemokine induced migration of human PBMC, particularly monocytes / macrophages. Furthermore, TWEAK promotes kidney infiltration of inflammatory cells and stimulates proliferation of kidney cells in vitro and in vivo. Thus, TWEAK may play role in the development of glomerulonephritis by promoting a local inflammatory environment and inducing kidney cells proliferation. Blocking TWEAK/Fn14 interaction may be a promising therapeutic strategy for the treatment of lupus nephritis.

The present study showed an inverse correlation between serum TWEAK and C3, C4, and a positive correlation with anti-dsDNA, SLEDAI and rSLEDAI, however no significant differences were found between patients with high activity and low activity.

SLE remains the archetype of immune complex– mediated disease, in which glomerular injury is initiated by the glomerular deposition of immune complexes and complement, triggering a cascade of inflammatory events. Although autoantibody deposition in the kidneys is a well-recognized and important pathogenic component of lupus nephritis, it is increasingly accepted that signals from the innate immune system and cellular immunity also contribute to the renal disease, since B cells, T cells, macrophages, and dendritic cells are also recruited into the inflamed kidney (Couzi *et al.,* 2007).

The present study showed significantly higher level of CD8 and lower levels of CD4 and a significantly decreased ratio of CD4 /CD8 in SLE patients than controls. Also, CD4/CD8 ratio correlated positively with C3 and negatively with antidsDNA, anti-C1q, sCD40L, TWEAK and SLEDAI, however no significant differences were found between patients with or without LN. This in agreement with Bakke *et al.,* (1983), who reported decreased CD4+ cells and CD4/CD8 ratio, and relatively increased CD8+ cells. Also, Keskin *et al.*, (1996), reported that, CD4+ cell subset was decreased both in active and inactive patients and CD8+ cell subset was increased in SLE patients compared to the control group, and no differences in CD4/CD8 ratio between active and inactive SLE patients. The preliminary results of Matsushita *et al.,* (2000), showed decreased CD4/CD8 ratio in SLE patients and those patients had good response to treatment than others with normal or increased ratio.

CD8- cytotoxic T lymphocytes are characteristically increased in the peripheral blood of SLE patients and this T lymphocyte subset activation was mainly type I IFN–dependent and that these cells were able to produce large amounts of nuclear-containing bodies in vitro, and correlated with disease activity, (Blanco *et al.,* 2005). In another study, Couzi *et al.,* (2007), reported that CD8- T lymphocytes are the main cells that infiltrate the periglomerular region in the kidneys of patients with lupus nephritis. In addition, this infiltrate appears to be specifically correlated with the presence of cellular crescents, Bowman's capsule rupture, and a poor response to conventional therapy. Moreover, patients who have a high histopathologic index of renal activity, renal failure, nephrotic syndrome, and hematuria have the more important renal CD8- T lymphocyte infiltrate supporting the idea that a new pathway, which is mediated by CD8- T lymphocyte effector cells, could be implicated in the development of lupus nephritis.

The correlation studies of studied serum biomarkers with kidney functions test, anti-

C1q antibodies and sCD40L showed positive correlation with serum urea, creatinine, proteinuria, while TWEAK correlated with proteinuria in addition to serum urea. In agreement with our results, Sinico *et al.*, (2005), found a positive correlation between anti-C1q antibodies and proteinuria while Marto *et al.*, (2005), did not find any correlation.

In conclusion, patients with SLE had high levels of anti-C1q, sCD40L and TWEAK, especially in LN than non-LN patients. Increased serum TWEAK levels had the highest sensitivity for development of LN in SLE patients. The presence of high levels serum TWEAK, anti-C1q, and sCD40L positively correlated with traditional disease activity parameters and may be used as an indicator for monitoring disease activity and renal involvement.

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