VALUE OF TUMOR NECROSIS FACTOR-LIKE WEAK INDUCER OF APOPTOSIS AND CHEMOKINE CXC LIGAND 13 AS BIOMARKERS FOR DISEASE ACTIVITY IN SYSTEMIC LUPUS ERYTHEMATOSUS

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\textbf{ABSTRACT}

Tumor necrosis factor -like weak inducer of apoptosis (TWEAK) triggers multiple cellular activities in a wide variety of cells, ranging from proliferation to cell death. It also causes upregulation of chemokine (C-X-C motif) receptor 5, and its ligand, chemokine (C-X-C motif) ligand 13 (CXCL13). However, the precise roles of TWEAK and CXCL13 in the pathogenesis of SLE and their association with disease activity still obscure. The study included forty SLE patients and twenty control subjects. SLE disease activity was evaluated by Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) 2000 score. Anti-dsDNA antibodies, serum complement C3, high-sensitivity CRP (hsCRP) concentrations, TWEAK and CXCL13 serum concentrations were detected by ELISA. TWEAK mRNA expression in peripheral blood mononuclear cells was estimated by relative quantitative Real Time-PCR. Serum concentrations of TWEAK and CXCL13 were significantly elevated in SLE patients compared to controls (p < 0.05) with significant increase in patients with active SLE compared to those with inactive disease (p < 0.05), they also significantly correlated with SLEDAI score and anti-dsDNA antibodies. TWEAK mRNA levels increased significantly in active lupus compared to inactive disease. It can be concluded that TWEAK serum and expression levels together with its inducible chemokine CXCL13 serum levels may benefit as biomarkers for prediction of
SLE disease activity, as well as possible targets for personalized therapies due to their involvement in the pathogenesis of SLE.

**Key words:** Systemic lupus erythematosus (SLE); tumor necrosis factor-like weak inducer of apoptosis (TWEAK); (C-X-C motif) ligand 13 (CXCL13).

**INTRODUCTION**

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease that involves disturbances in both innate and adaptive immune mechanisms, including complex interactions between T lymphocytes, B lymphocytes and other antigen-presenting cells (Azevedo et al., 2014). Cytokines play important roles in modulating both immune responses. A fine balance among cytokines is required to maintain immune homeostasis (Ohl and Tenbrock, 2011). Cytokine-mediated immunity could contribute to the pathogenesis of SLE and other autoimmune diseases (Lourenço and La Cava, 2009).

Tumor necrosis factor-like weak inducer of apoptosis (TWEAK) is a recently identified proinflammatory cytokine belonging to the TNF superfamily that functions through binding to its receptor, fibroblast growth factor-inducible 14 (Fn14), in target cells (Burkly, 2014). TWEAK gene is located at chromosome 17; the intracellular domain of TWEAK contains a putative serine phosphorylation site. The extracellular domain contains the receptor-binding site (Cheng et al., 2013). TWEAK is a cell surface-associated type II transmembrane glycoprotein (30 kD) that circulates in plasma as a soluble form (sTWEAK) with a molecular weight of 18 kD (Winkles, 2008). It is widely expressed and can be found in pancreas, intestine, heart, brain, lung, ovary, the vasculature, skeletal muscle, liver, and kidney (Bertin et al., 2013).

Binding of TWEAK to its receptor mediates multiple biologic effects such as cellular growth, proliferation, migration, differentiation, apoptotic cell death, inflammation, and angiogenesis (Burkly et al., 2007). In addition, TWEAK induces the expression of different cell adhesion molecules and proinflammatory cytokines through activation of the nuclear factor-κB pathway (Roos et al., 2010) and mitogen-activated protein kinases pathways (Wang et al., 2010).

Accordingly, TWEAK has a prominent role in the development of chronic autoimmune disease, the pathogenesis of
renal, vascular injury and neuropathy (Ortiz et al., 2009). Although the expression of TWEAK in peripheral blood mononuclear cells (PBMCs) has been recently observed in the patients with multiple sclerosis (Desplat-Jégo et al., 2009), TWEAK expression in PBMCs in SLE, as well as its functional significance in T cell and B cell activation remain unclear.

On the other hand, TWEAK stimulation of glomerular mesangial cells resulted in upregulation of (C-X-C motif) receptor 5 (CXCR5), and its ligand, chemokine (C-X-C motif) ligand 13 (CXCL13), which is also known as B-cell-attracting chemokine-1 (Campbell et al., 2006). The chemokine CXC ligand 13 protein (CXCL3) is a potent B-cell chemoattractants. In SLE, it is responsible for the accumulation of inflammatory cells in the kidneys as it induces formation of ectopic lymphoid tissues (Schiffer et al., 2009). Moreover, it might participate in exacerbation of lupus nephritis through promotion of local activation of T and B cells in these ectopic lymphoid tissues (Segerer and Schlondorff, 2008). However, the significance of serum CXCL13 as a noninvasive biomarker for SLE activity and its correlation with other parameters of disease activity still need further investigation.

Therefore, this study aimed to assess the serum level and the expression pattern of TWEAK in PBMCs in conjunction with the serum level of CXCL13 in patients with SLE in order to throw light on their crosstalk or interplay as contributors in the molecular pathogenesis of SLE and to assess their potential value as diagnostic and prognostic markers.

**MATERIALS AND METHODS**

This study was carried out on 60 subjects; divided into two groups; Group I: included 40 premenopausal SLE female patients selected from the Outpatient Clinic of the Rheumatology and Rehabilitation Department, Tanta University Hospitals, Egypt. In addition, 20 healthy volunteers matched for age and sex were recruited for participation as control group II. The American College of Rheumatology (ACR) revised criteria for the classification of SLE (Hochberg et al., 1997) was used for diagnosis of SLE and selection of cases. Subsequently, the SLE disease activity index (SLEDAI) 2000 was used for the determination of disease activity in the selected
cases. This index constitutes a range of clinical and laboratory findings of 24 descriptors in nine organ systems over the preceding 30 days as skin rash, alopecia, mucosal ulcers and proteinuria. Descriptors of SLEDAI are documented as present or absent. Each of the descriptors has a weighted score and the total score of SLEDAI is the sum of all 24 descriptor scores. The total SLEDAI score falls between 0 and 105, with higher scores representing higher disease activity (Gladman et al., 2002). Patients were then classified into two subgroups: Group I (A): included 20 patients with active SLE who were having a SLEDAI score of \( \geq 10 \); and Group I (B): included 20 patients with inactive SLE who were having a SLEDAI score of less than 10 at the time of the study.

The study protocol was approved by the Local Research Ethics Committee at Tanta University, and written informed consent was obtained from each participant. SLE patients who were receiving any immune-modulatory therapy for at least one month before blood sampling were excluded from this study. All studied groups were subjected to thorough history taking and clinical examination.

**Blood sampling:** After 12 hours of overnight fasting, 7ml of venous blood samples were taken from every investigated subject, and distributed in two dry sterile untreated and EDTA-treated centrifuge tubes. The blood in the untreated tube was allowed to clot at room temperature. Both tubes were centrifuged at 2000 rpm for 10 minutes, plasma and serum were separated and stored at \(-70 \, ^\circ \text{C}\) until the time of analysis.

**Laboratory investigations:** Both patients and control groups were subjected to the following investigations:

1) Double stranded (DNA) (anti-dsDNA) by ELISA Kit (ZEUS scientific Inc., USA.) (Hanaoka et al., 2012).

2) Serum complement C3 was measured by AssayMax Human C3 complement ELISA kit (EC2101-1) (Li et al., 2013).

3) Plasma high-sensitivity CRP (hsCRP) was measured using Diamed Eurogen CRP ELISA kit (Firooz et al., 2011).

4) Serum soluble TWEAK Level was measured using a commercially available ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions (Liu et al., 2011).

5) Serum CXCL13 level was quantified by commercially available Quantikine ELISA methodology (R&D Systems,
Hanaa Hibishy Gaballah, Noha M. Shafika et all.

Minneapolis, MN, USA) according to the manufacturer’s instructions (Wong et al., 2010).

6) Estimation of TWEAK mRNA expression in Peripheral blood mononuclear cells (PBMCs):

A) Preparation of PBMCs: Peripheral blood mononuclear cells were prepared by density gradient centrifugation using Ficoll-Hypaque (Pharmacia, Uppsala, Sweden). Briefly, heparinised blood was carefully layered on Ficoll, and PBMCs were harvested from the white interphase after centrifugation for 30 minutes at 400 x g, at room temperature and washed with phosphate buffered saline (PBS) (Slade et al., 1988). The PBMCs samples were stored at −80°C till the samples were further processed for RNA isolation.

B) RNA extraction, cDNA synthesis and Real time PCR:

i. RNA extraction: Total RNA was isolated from frozen PBMCs samples by Qiagen RNeasy Mini Kit according to the protocol supplied by the manufacturer. RNA was eluted and its concentration was measured spectrophotometrically (280).

ii. cDNA synthesis: The extracted RNA was reverse transcribed into cDNA using (High capacity cDNA synthesis kit, Applied Biosystems). Ten μl of random hexamer primers (Roche, Mannheim, Germany) were added to 21 μl of RNA which was denatured for 5 minutes in the thermal cycler (Biometra, USA). The RNA-primer mixture was cooled to 4°C. The cDNA master mix was prepared (5 μl of first strand buffer, 10 mM of dNTPs, 1 μl of RNase inhibitor, 1 μl of reverse transcriptase Superscript™ II-RT enzyme and 10 μl of DEPC treated water ) according to the kit protocol and was added to each sample. The total volume of the cDNA master mix was 19 μl for each sample. This was added to 31 μl RNA-primer mixture resulting in a reaction volume of 50 μl, which was then incubated in the programmed thermal cycler one hour at 37 °C, followed by inactivation of enzymes at 95 °C for 10 minutes, and finally cooled at 4°C. The RNA was reverse transcribed into cDNA which was then stored at -20°C.

iii. Real-time quantitative PCR: One μl of the cDNA was added to a 20 μl reaction mixture of the QuantiTect SYBR-Green PCR kit (Qiagen) and 0.5 μM from the specific primer pair for human TWEAK. This cDNA was then amplified using the Step One instrument (Applied Biosystems, USA) as follows: Initial denaturation at 95°C for 15 minutes was followed by 40 cycles with denaturation at
95°C for 15 sec, annealing at 60°C for 30 sec and extension at 72°C for 20 sec. A control reaction without a DNA template was performed in parallel to detect genomic DNA contamination. Primer sequences specific for the TWEAK were designed according to Peng et al. (2014) as follows: sense, 5’ CCCTGCACGTGCCTGGAGGAA 3’, and anti-sense, 5’ AGACCAGGGCCCTCAGTGA 3’. Primers for GAPDH were included as an internal control: sense, 5` TG AAGGTCGAGTCAACGGATTTGGT 3`, and antisense, 5` CATGTGGGCCATGAGGTCCACCAC 3`.

The determination of the relative levels of gene expression was performed using the cycle threshold (△△Ct) method and normalized to the housekeeping gene GAPDH, which was not altered by the experimental conditions.

Statistical Analysis

Analyses were performed using the SPSS software version 20.0 (SPSS Inc, Chicago, IL). Baseline characteristics are presented as mean ± standard deviation for the continuous variables. Comparisons between groups were conducted using ANOVA test. The correlation between the studied clinical and biochemical parameter was calculated using Pearson’s correlation coefficient. Multiple logistic regression analysis was used to estimate the odds ratios (ORs) and 95% confidence intervals (CI) for the studied parameters. Receiver operating characteristics (ROC) analysis was used to identify the optimal threshold values of the studied parameters.

RESULTS

Table 1 showed the clinical and laboratory characteristics of the studied groups; There were no significant differences in age and sex between SLE patients and controls. TWEAK mRNA levels in SLE patients (0.74±0.18) were significantly higher than controls (0.34±0.06, p < 0.01), with significantly higher levels in patients with active disease (0.90±0.06) compared to those with inactive disease (0.56±0.05, p < 0.01). Meanwhile, the serum concentrations of TWEAK in SLE patients (3.10±0.76 ng/ml), were significantly higher than those in controls (1.12±0.37 ng/ml, p<0.001), with being significantly higher in active SLE (3.70.3±0.44 ng/ml) compared to inactive disease (2.47±0.42 ng/ml, p<0. 01). Serum CXCL13 level was significantly higher in SLE patients (191.6+36.5 pg/ml) compared
to controls (36.37±16.2 pg/ml, p < 0.01), with significantly higher levels in patients with active SLE (281.1±82.2 pg/ml) than those with inactive disease (97.9±32.7 pg/ml, p < 0.01). In comparison with control values, both groups of SLE patients demonstrated significantly increased serum concentrations of hsCRP and anti-dsDNA antibody.

Using Pearson correlation test, TWEAK mRNA and serum levels showed significant positive correlations with the levels of SLEDAI score, serum concentrations of CXCL13, and anti-dsDNA antibody. On the other hand, significant negative correlations were found between both TWEAK mRNA and serum levels and serum C3 complement level. Likewise, serum concentrations of CXCL13 exhibited significant positive correlations with all the studied parameters except for C3 complement level which showed a significant negative correlation (Table 2).

Table (3) showed forward stepwise multiple logistic regression analysis of factors that might be independently associated with SLE disease activity. It was performed on a number of predictors including serum and expression levels of TWEAK as well as serum levels of CXCL-13 as independent variables, and the SLEDAI as the dependent variable. It was found that serum CXCL-13 followed by serum then expression levels of TWEAK were the most important predictors of SLE disease activity.

Receiver operating characteristics (ROC) analysis was used to evaluate the diagnostic value of TWEAK mRNA, serum TWEAK and CXCL13 and to identify the optimal cut off values. Sensitivity and specificity, positive and negative predictive values of each parameter were profiled by curves (figure1). The area under the curve can range from 0.5 to 1 and diagnostic tests that approach 1 indicate a perfect discriminator. Concomitantly, the optimal cut off value of serum CXCL13 was 161 pg/ml, the sensitivity at this cut off point was 95.6%, the specificity was 94.2%, the positive predictive value (PPV %) was 96.6% and the negative predictive value (NPV %) was 92.5% and the area under the curve was 0.94. Furthermore, the optimal cut off value of serum TWEAK was 3035 pg/ml, the sensitivity at this cut off point was 94.7%, the specificity was 92.2%, the positive predictive value (+PV %) was 90.7% and the negative predictive value (–PV %) was 89.6% and the area under the curve was 0.89. Data are summarized in table (4).
Table (1): Clinical and laboratory characteristics of the SLE patients and healthy controls.

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=20)</th>
<th>Active SLE (n=20)</th>
<th>Inactive SLE (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>30.4 ±0.37</td>
<td>29 ±0.22</td>
<td>31±0.43</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>-</td>
<td>5.24±1.8</td>
<td>2.17±0.8</td>
</tr>
<tr>
<td>SLEDAI</td>
<td>-</td>
<td>14.7±2.9</td>
<td>4.49±1.3</td>
</tr>
<tr>
<td>TWEAK mRNA</td>
<td>0.34±0.06</td>
<td>0.9±0.06</td>
<td>0.56±0.05</td>
</tr>
<tr>
<td>Serum TWEAK (ng/ml)</td>
<td>1.12±0.37</td>
<td>3.70±0.44</td>
<td>2.47±0.42</td>
</tr>
<tr>
<td>CXCL13 (pg/ml)</td>
<td>36.37±16.2</td>
<td>281.1±82.2</td>
<td>97.9±32.7</td>
</tr>
<tr>
<td>hsCRP, (mg/l)</td>
<td>3.1 ± 1.94</td>
<td>11.54 ± 2.5</td>
<td>9.9±1.73</td>
</tr>
<tr>
<td>Anti-dsDNA</td>
<td>12.9±8.15</td>
<td>172.5±64.1</td>
<td>42.1±9.61</td>
</tr>
<tr>
<td>C3 (mg/dl)</td>
<td>128.23±32.42</td>
<td>92.81±40.18</td>
<td>108.29±29.62</td>
</tr>
</tbody>
</table>

Significance =P <0.05
Data are presented as the mean ±SD. a significant difference between active& inactive; b significant difference between active& controls; c significant difference between inactive& controls. SLEDAI, Systemic Lupus Erythematosus Disease Activity Index ;TWEAK, Tumor necrosis factor -like weak inducer of apoptosis; CXCL13, CXC ligand 13 protein; hs-CRP, high sensitive-CRP; dsDNA ,Double stranded (DNA); C3, complement component 3.

Table (2): Correlation analysis between the different studied variables in patients with active and inactive SLE.

<table>
<thead>
<tr>
<th>Variables</th>
<th>r_b</th>
</tr>
</thead>
<tbody>
<tr>
<td>TWEAK mRNA and disease duration</td>
<td>0.49*</td>
</tr>
<tr>
<td>TWEAK mRNA and SLEDAI</td>
<td>0.58*</td>
</tr>
<tr>
<td>TWEAK mRNA and anti-dsDNA</td>
<td>0.49*</td>
</tr>
<tr>
<td>TWEAK mRNA and C3 (mg/dl)</td>
<td>-0.58*</td>
</tr>
<tr>
<td>TWEAK mRNA and serum TWEAK(ng/ml)</td>
<td>0.65*</td>
</tr>
<tr>
<td>TWEAK mRNA and serum CXCL13 (pg/ml)</td>
<td>0.54*</td>
</tr>
<tr>
<td>Serum TWEAK (ng/ml) and disease duration</td>
<td>0.47*</td>
</tr>
<tr>
<td>Serum TWEAK (ng/ml) and SLEDAI</td>
<td>0.61*</td>
</tr>
<tr>
<td>Serum TWEAK (ng/ml) and anti-dsDNA</td>
<td>0.59*</td>
</tr>
<tr>
<td>Serum TWEAK (ng/ml) and C3 (mg/dl)</td>
<td>0.47*</td>
</tr>
<tr>
<td>Serum TWEAK (ng/ml) and serum CXCL13</td>
<td>0.57*</td>
</tr>
<tr>
<td>Serum CXCL13 (pg/ml) and disease duration</td>
<td>0.64*</td>
</tr>
<tr>
<td>Serum CXCL13 (pg/ml) and SLEDAI</td>
<td>0.59*</td>
</tr>
<tr>
<td>Serum CXCL13 (pg/ml) and anti-dsDNA</td>
<td>0.45*</td>
</tr>
<tr>
<td>Serum CXCL13 (pg/ml) and C3 (mg/dl)</td>
<td>0.78*</td>
</tr>
</tbody>
</table>

r= Pearson’s correlation coefficient, *Significant at p <0.05; SLEDAI , Systemic Lupus Erythematosus Disease Activity Index ;TWEAK, Tumor necrosis factor -like weak inducer of apoptosis; CXCL13, CXC ligand 13 protein; hs-CRP, high sensitive-CRP; dsDNA ,double stranded DNA; C3, complement component 3.
Table (3): Logistic regression analysis with the background elimination method using the 3 studied parameters as independent variables, and SLEDAI as the dependent variable.

<table>
<thead>
<tr>
<th>Variables</th>
<th>B</th>
<th>S.E.</th>
<th>P-value</th>
<th>OR</th>
<th>95.0% C.I. for odd</th>
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<td></td>
<td></td>
<td>Lower</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Upper</td>
</tr>
<tr>
<td>Serum CXCL13 (pg/ml)</td>
<td>1.56</td>
<td>1.32</td>
<td>0.03</td>
<td>2.8</td>
<td>1.99</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.54</td>
</tr>
<tr>
<td>Serum TWEAK (ng/ml)</td>
<td>2.82</td>
<td>1.35</td>
<td>0.04</td>
<td>1.9</td>
<td>1.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.55</td>
</tr>
<tr>
<td>TWEAK mRNA</td>
<td>2.37</td>
<td>2.58</td>
<td>0.04</td>
<td>1.5</td>
<td>1.33</td>
</tr>
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<td></td>
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<td>5.87</td>
</tr>
</tbody>
</table>

B: regression coefficient; S.E: Standard error; OR: Odds ratio; CI: confidence interval; TWEAK: Tumor necrosis factor-like weak inducer of apoptosis; CXCL13: CXC ligand 13 protein.

Table (4): The performance characteristics for serum TWEAK and CXCL13 with active and inactive SLE.

<table>
<thead>
<tr>
<th></th>
<th>Cutoff</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>serum CXCL13 (pg/ml)</td>
<td>&gt; 161</td>
<td>95.6%</td>
<td>94.2%</td>
<td>96.6%</td>
<td>92.5%</td>
<td>0.94</td>
</tr>
<tr>
<td>serum TWEAK (ng/ml)</td>
<td>&gt; 3.03</td>
<td>94.7%,</td>
<td>92.2%,</td>
<td>90.7%</td>
<td>89.6%</td>
<td>0.89</td>
</tr>
</tbody>
</table>

PPV: positive predictive value, NPV: negative predictive value, TWEAK: Tumor necrosis factor-like weak inducer of apoptosis; CXCL13:CXC ligand 13 protein.
Figure (1): ROC curves for serum levels of TWEAK and CXCL13.

DISCUSSION

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by overproduction of autoantibodies, tissue deposition of immune complexes, and high levels of inflammatory cytokines resulting in a systemic pro-inflammatory state (Frieri, 2013). As a member of the TNF-ligand superfamily, TWEAK plays a major role in stimulation of cell growth and angiogenesis, induction of inflammatory cytokines, and stimulation of apoptosis (Zhu et al., 2012); its dysregulated expression has been recently implicated in the pathogenesis of many chronic inflammatory and autoimmune diseases. Diverse lines of evidence suggest that co-expression of TWEAK and its receptor, fibroblast growth factor-inducible (Fn14), has a role in the promotion of joint destruction and vascular proliferation (Kamijo al., 2008) as well as replacement of damaged bone and cartilage in RA (Dharmapatni et al., 2011). Besides, TWEAK contributes to the new vessel formation and proliferative angiopathy seen in systemic sclerosis -related pulmonary arterial hypertension or renal crisis (Nazeri et al., 2014). Moreover, TWEAK might be involved in the etiopathogenesis of SLE through induction of several proinflammatory cytokines and chemokines on the one hand, and on the other hand by mediating the autologous monocyte death induced by lupus T cells (Kaplan et al., 2002).

Among the TWEAK-inducible chemokines, chemokine (C-X-C motif) ligand 13 (CXCL13) is a major regulator of B1 and B2 cell
trafficking, it is responsible for activation of autoreactive T helper cells and autoantibody production in target organs during the development of lupus (Wong et al., 2010). Reliable lupus biomarkers are needed to help early detection of flare, distinction between flare and chronic damage, distinction between flare and infection and monitoring response to therapy (Adhya et al., 2011). Therefore, this study aimed to gain biological insight on the role of TWEAK and CXCL13 in the pathogenesis of SLE and to assess their value as potential diagnostic and prognostic biomarkers.

The current study revealed that TWEAK mRNA expressions in total PBMCs from SLE patients are significantly higher than those from controls with significantly higher levels in patients with active lupus compared to those with inactive disease. These findings are in agreement with the results from earlier studies by Liu et al. (2011) who concluded that TWEAK mRNA expression was pronounced in total PBMCs from SLE patients particularly those with the active form of the disease. Also they demonstrated that the activation of TWEAK may not only be functionally enhanced in SLE, but might also be a process specific to SLE. Moreover, Kaplan et al. (2002) reported an upregulated TWEAK expression on activated lupus T cells which is associated with autologous monocyte death.

On the other hand, Wang et al. (2012) reported decreased TWEAK expression levels in Chinese SLE patients as compared to controls. This inconsistent finding may be attributed to the different characteristics of the studied cohort of patients as regards age, ethnicities and the extent of renal involvement. TWEAK mRNA expression was found to be downregulated in experimental studies concomitantly as mouse age advances and nephritis become evident (Chicheportiche et al., 2000), thus lending support to our rationale. However, verification on large scaled studies is warranted.

Concomitantly, the serum TWEAK has shown significantly increased levels in SLE patients compared to controls, with significantly higher levels in patients with active lupus than those with inactive disease. These findings are in line with the study by ElGendi and El-Sherif (2009) and who reported elevated serum TWEAK in active lupus relative to inactive lupus. Therefore, it can be concluded that TWEAK induces the production of other proinflammatory cytokines and chemokines which propagate tissue inflammatory injury in SLE (Gao et al. 2009).
The current study demonstrated that TWEAK mRNA expression and serum levels showed significant positive correlation with each other. Conceivably, this correlation might be explained by the presence of an accelerated translational machinery to cope with the active transcription of TWEAK gene resulting in increased production of circulating TWEAK protein in SLE patients.

In addition, TWEAK mRNA expression and serum levels were positively correlated with SLEAI score and anti-dsDNA titres and negatively correlated with serum C3 complement level. Consequently, these findings support the notion that the activation of TWEAK is functionally enhanced in SLE. Hence it plays a crucial role in the pathogenesis and aggravation of SLE as a proinflammatory cytokine, thus can potentially serve as a helpful biomarker in the clinical follow-up.

Of note, serum CXCL13 level in the present study was significantly higher in SLE patients compared to controls with significantly higher levels in active SLE compared to inactive disease; it was also significantly correlated with anti-dsDNA titres and SLEAI score. These results may indicate that the aberrant B-cell trafficking caused by altered expression of CXCL13 is involved in aggravation of SLE. These findings are in agreement with the study by Wong et al. (2010) who showed that Plasma CXCL13 concentrations were significantly higher in SLE patients than controls and the increased level of CXCL13 is a feature of SLE that correlates positively and significantly with disease activity. Accordingly, it can be concluded that CXCL13 might be a readily available marker for monitoring the extent of aberrant B-cell dysfunction in SLE; this is because of the fact that CXCL13 can induce the trafficking of CXCR5+ T lymphocyte subset designated as follicular helper T lymphocytes which are specifically involved in autoantibody production. Supporting these findings, Schiffer et al. (2008) reported that CXCL13 was one of only a few inflammatory markers that were expressed in the kidney at an early point of disease, suggesting a possible pathogenic role for disease manifestation. Moreover, Schiffer L et al., 2009 has shown that excessive expression of dendritic cell-derived CXCL13 is a distinctive early event for nephritis in a murine model of systemic lupus erythematosus.

Well in line, circulating CXCL13 has recently been proposed as a potential marker of disease activity in patients with rheumatoid
arthriti(s) ([Rioja et al., 2008]), and excess levels of circulating CXCL13 correlated with active cutaneous vasculitis in Hepatitis C virus-related cryoglobulinaemia ([Sansoono et al., 2008]), which suggest a common role for CXCL13 in this wide variety of diseases. Moreover, altered expression of the chemokine receptor-ligand pair, CXCR5/CXCL13 was found to participate in the pathogenesis of B cell chronic lymphocytic leukemia ([Bürkle et al., 2007]) and B-cell dysfunctions during HIV-1 infection ([Cagigi et al., 2008]).

In conclusion, this study revealed that TWEAK serum and expression as well as serum CXCL13 differs significantly between SLE patients and controls which supports the viewpoint that TWEAK plays a key role in the pathogenesis of SLE through activation of multiple down-signaling pathway, inducing proinflammatory cytokines and chemokines including B-cell-attracting chemokine-1 or CXCL13 and by affecting cell proliferation/ apoptosis. These multiple contribution to the pathogenesis of SLE suggest that TWEAK should be considered as a potential target for the development of novel therapeutics for SLE. TWEAK and CXCL13 were also positively correlated with anti-dsDNA titres and with SLEDAI score; so they may be useful as potential biomarkers for effective assessment of disease activity, which may help in the clinical management of the disease and in the measurement of response to therapy.

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VALUE OF TUMOR NECROSIS FACTOR-LIKE WEAK ……..


VALUE OF TUMOR NECROSIS FACTOR-LIKE WEAK


الملخص العربي

قيمة عامل نخر الورم المحفز الضعيف للموت المبرمج للخلايا مع المجند الثالث عشر من الكيموكين سى أكس سى كمؤشرات حيوية محتملة للنشاط المرضي في مرضى الذبابة الحمراء

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تطرق تأثير الورم المحفز الضعيف للموت المبرمج للخلايا (تي ديبيل أي أي كى) الأشعة الضوئية المتعددة في مجموعة واسعة من الخلايا، بدءًا من تواجدها إلى مقتادها. كما أنه يقوم بتنظيم عمل مستقبلات الكيموكتين من نوع سى أكس سى أر 5 والجزء القادم منه والذي يسمى سى أكس سى أر 13. عند النظر إلى تأثير ديبيل أي وكذلك بروفور مسي أكس سى أر 13 في بانولوجيا مرض الذبابة الحمراء وارتباطهم بنشاط المرض لا تزال غامضة. في هذا البحث تم اتخاذ أربعين مريض بالإصابة الحمراء وعشرين فردًا كعينة ضابطة لهذه الدراسة. تم قياس نشاط المرض بواسطة مؤشر نشاط مرض الذبابة الحمراء 2000 درجة وتم قياس مستوى الأجسام المضادة للإيلب المذود والبروتين المصاحب الثالث وبروتينات سى التفاعلي العالي الحساسية وكذلك مركبين تي ديبيل أي أي كى وسى أكس سى أر 13 بواسطة تقنية الألبرة التعبير الجيني عامل نخر الورم المحفز الضعيف للموت المبرمج للخلايا في الخلايا الدموية المحيطية وحدود النواة تم تعينة بواسطة تقنية تفاعل البلمرة التسلسلية شبة الكمي. تشير نتائج هذا البحث أن تركز مستوى تي ديبيل أي أي كى وسى أكس سى أر 13 في المصل كانت مرتفعة بشكل ملحوظ في مرضى الذبابة الحمراء مقارنة مع العينة الضابطة مع زيادة كبيرة ذات دلالة في المرضى الذين يعانون من مرض الذبابة الحمراء النشط مقارنة مع الذين يعانون من المرض غير النشط. والنهاية السابقة مرتبطة بالنشاط شائع بالمرض ومستوى الأجسام المضادة للإيلب المذود. ولاحظ هذا أن تأثير الجيني ديبيل أي أي كى في الخلايا الدموية المحيطية وحدود النواة ازداد زيادة ذات دلالة في المرض النشط مقارنة بالمريض غير النشط. وبناء على النتائج التي وصلنا إليها في هذا البحث؛ نستخلص أن مستوى تي ديبيل أي أي كى وسى أكس سى أر 13 في المصل وتعبير الجيني يمكن اعتباره مصريات حيوية للتنبؤ بوجود مرض الذبابة الحمراء النشط وقد تمكنت الحيوية يمكن أن تكون هدف للمعاقب في علاج هذا المرض.