

# Assessment of Tumor Necrosis Factor Related Weak Inducer of Apoptosis (TWEAK) level in Systemic Lupus Erythematosus patients (nephritis patients)

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**Abstract.** Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease characterized by the production of antibodies to components of the cell nucleus in association with a diverse array of clinical manifestations. The exact pathoetiology of SLE remains elusive. The aim of the current study were to detect the analytic estimation of serum TNF-like weak inducer of apoptosis (TWEAK) level in Systemic Lupus Erythematosus patients (nephritis patients). Methods: This investigation was conducted at Najaf public laboratories and Biological Therapy center in Marjan Teaching Hospital in Babylon, Iraq. During the period from October, 2022 till May, 2023. This investigation was included 120 subjects women divided into two groups 90 patients group (all patients 90 has ANA positive , 60 only from these women has positive anti-double stranded antibody suffering from SLE) that age ranges from 20-39 years and control group was composed of 30 female healthy persons. Parameters that estimate in this investigation include Creatinine concentration and TNF-like weak inducer of apoptosis (TWEAK) estimate by using enzyme linked immune sorbent assay. As well as studying the relation between this biomarker level, Body Mass Index (BMI), age, and Hb and creatinine. Results: The results showed a significant a significant different (P-Value< 0.05) in Creatinine concentration, and significant increase (P-Value < 0.05) in level of TNF-like weak inducer of apoptosis (TWEAK) in systemic lupus erythematosus patients as comparison with healthy groups. Enzyme Linked Immune Sorbent Assay measure of serum TNF-like weak inducer of apoptosis (TWEAK) indicated more significant levels for systemic lupus erythematosus patients than healthy controls. Conclusion: The current study concluded that a TNF-like weak inducer of apoptosis (TWEAK) is a prognostic marker and early detection of systemic lupus erythematosus with renal disease associated with some complication.

## 1 Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease. The name lupus erythematosus refers to the facial skin rashes commonly found in SLE patients. It was the

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13th century physician Rogerius that first used the term lupus (Greek for wolf) erythematosus (Latin for red) to describe these facial lesions resembling a wolf bite [1]. The multifactorial interaction between genetic and environmental factors together with hormonal factors influence the development of the disease [2]. Antibodies to double stranded (ds) DNA are found in up to 70% of SLE patients at some point during the course of their disease, and are 95% specific for SLE, making them a valuable disease marker [3]. Renal involvement occurs in 40–70% of all SLE patients and is a major cause of morbidity and hospital admissions [4]. The normal procedure of waste disposal starts with the apoptotic cell which secretes “find-me” signals to recruit phagocytes, and then displays “eat-me” signals to facilitate recognition and ingestion of the dead cell (*i.e.* efferocytosis) [5]. The deposition of immune complexes in the kidney is the cause of the renal damage in LN [6]. Members of the tumor necrosis factor (TNF) superfamily of cytokines are typically expressed as type 2 transmembrane proteins with homologous TNF domains [7]. TWEAK is also the only member of the TNF family which can bind the cognate Fn14 receptor – the smallest member of TNF receptor superfamily – and trigger signalling which can lead to growth and proliferation, angiogenesis, and in an inflammatory scenario, stimulation of apoptosis [8]. CD163 is expressed exclusively on the cells of the monocytic–macrophage lineage and has been identified as the secondary, decoy receptor for TWEAK [9]. The promoter region has multiple nuclear factor-kappaB (NF- $\kappa$ B) binding sites, enabling positive feedback regulation between Fn14 and NF- $\kappa$ B [10]. TWEAK appears to be involved in the regulation of a multitude of genes; in vascular smooth muscle cells (VSMC) alone [11]. In addition to activating canonical and non-canonical NF- $\kappa$ B, TWEAK induces mitogen activated protein kinase (MAPK) and activator protein-1 (AP-1) signalling pathways [12]. TWEAK has a proinflammatory effect in adipocytes; however, this is mediated by NF- $\kappa$ B, and ERK pathways rather than JNK signaling [13]. TWEAK unlike TNF $\alpha$ , can be detected at higher expression levels and is expressed in several more tissues which include heart, brain, kidneys, and mononuclear blood cells [14].

## **2 Methods**

### **2.1 Subject population**

The study was directed in Najaf public laboratories and Biological Therapy center in Marjan Teaching Hospital in Babylon, Iraq. 120 subjects women were tested by the Antinuclear antibody and anti-double strand antibody test, 60 only from these women has positive anti-double stranded antibody suffering from SLE, the control group was composed of (30) healthy persons. The samples were gathered during the period from October, 2022 till May, 2023. The patients age and control rang (20 – 39) years. Patient and control group numerals were divided into groups by Hb concentration, age, and body mass index.

### **2.2 Exclusion criteria**

All other autoimmune diseases include (rheumatoid arthritis, multiple sclerosis, diabetes, pernicious anemia, graves’ disease, hashimota thyroiditis), blood disease, smoking, male with SLE and also other disease that are related to patient were excluded from the study.

## 2.3 Collection of blood samples

The blood samples were drawn from vein by sterilized syringes with 5 milliliters. The sample put in the two labeled tubes, first group of tubes contains EDTA as anti-coagulants to prevent clotting of blood to be used for hematological studies. The second group of tubes was without anti-coagulant as gel tubes, for blood to be used for preparing serum for following biochemical and biomarkers. Blood was left at room temperature for 10 minutes for clotting, centrifuged 6000 rpm for 10 minutes, and then serum was separated and freezing at -80 °C until time for performed the laboratory analysis for study.

## 2.4 Body Mass Index (BMI)

Electronic balance and height tool, were employed for account the weight and height, then applied the neutralization below:

BMI = Weight [kg]/Height [m<sup>2</sup>].

## 2.5 Biochemical Parameter

### 2.5.1 Measurements of serum creatinine concentration

The assessment of serum creatinine concentration were provided by using creatinine kit (Biolab. France) and measured manually by Spectrophotometer (Milton Roy Company, U.S.A).

## 2.6 Biomarker measurement

The assessment of serum Tumour Necrosis Factor Related Weak Inducer of Apoptosis (TWEAK) concentration is provided by (sunlong- china) sandwich immunoassay technique (enzyme linked immunosorbent assay-automated microtiter plate), ELISA reader (Bio kit SL1771HU).

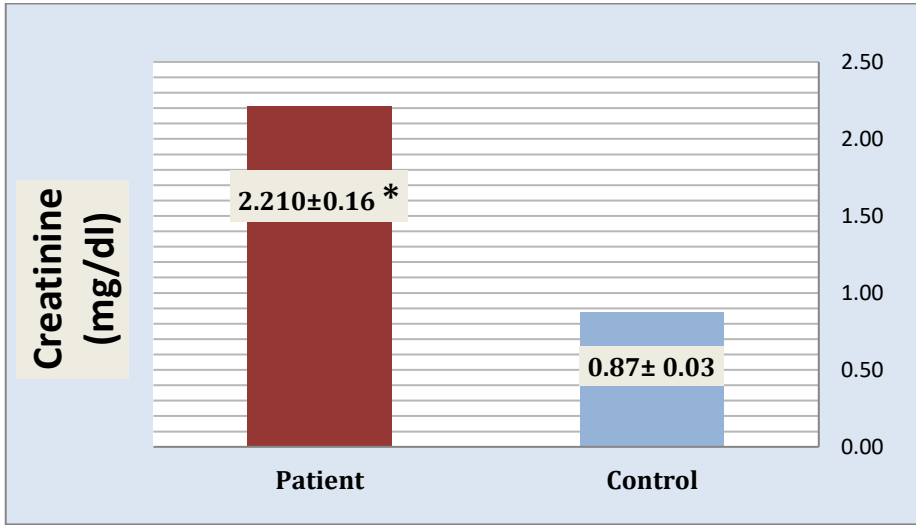
## 2.7 Statistical analysis

For statistical analysis, IBM-SPSS statistics 24 was used to test treatment responses versus controls. T-test and repeated measures ANOVA, and Standard Error were applied to test the variability and the statistical significance of this experiment. In addition. Treatment effects are considered statistical significant as P value < 0.05. For creation the plots and tables, we used SigmaPlot 9.0 software, and Microsoft excel [15, 16].

# 3 Results

## 3.1 Creatinine concentration

The statistical analysis in figure 1 revealed a significant different (P-Value< 0.05) in serum level of creatinine, there is an increase in patients (**2.210±0.1**) mg/dl compared with that in control (**0.87± 0.03**).



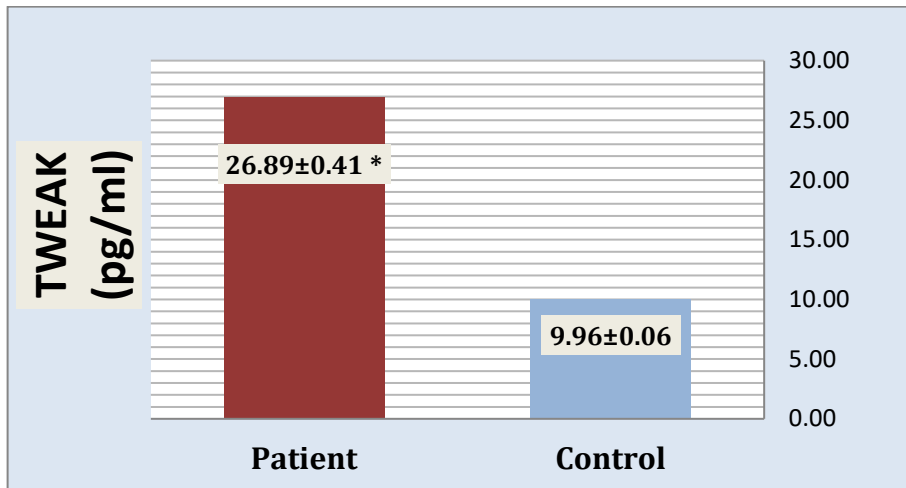
**Fig. 1.** Creatinine concentration (mg/dl) in control and systemic lupus erythematosus patients.

\* refer to significant differences (P-Value < 0.05)

Patients n=60, control n=30

### 3.2 Tumour Necrosis Factor Related Weak Inducer of Apoptosis (TWEAK) level in systemic lupus erythematosus patients in comparison with control (healthy) group

TWEAK level in studied groups is shown in figure (2). The results are indicated that a significant increase (P-Value < 0.05) in mean level of TWEAK in patients with systemic lupus erythematosus (26.89 ± 0.41) pg/ml, in comparison with that mean of control group (9.96 ± 0.06) pg/ml.



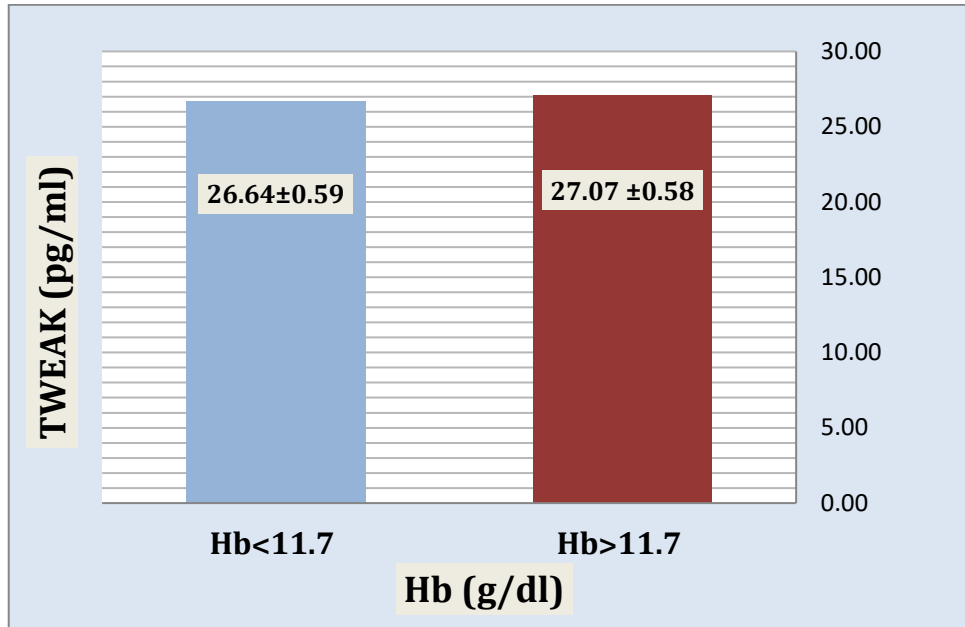
**Fig. 2.** TWEAK (pg/ml) level in patients with systemic lupus erythematosus and control groups.

\* refer to significant differences (P-Value < 0.05)

Patients n=60, control n=30

### 3.3 Comparison of biomarker in systemic lupus erythematosus patients according to Hemoglobin concentration: Tumour Necrosis Factor Related Weak Inducer of Apoptosis (TWEAK)

Serum TWEAK level in both Hb groups of systemic lupus erythematosus patients as shown in figure (3), which referred a no significant difference (P-value >0.05) in TWEAK level between group of Hb less than 11.7 (27.07±0.58) pg/ml and group of Hb more than 11.7 (26.64±0.59) pg/ml of systemic lupus erythematosus patients.



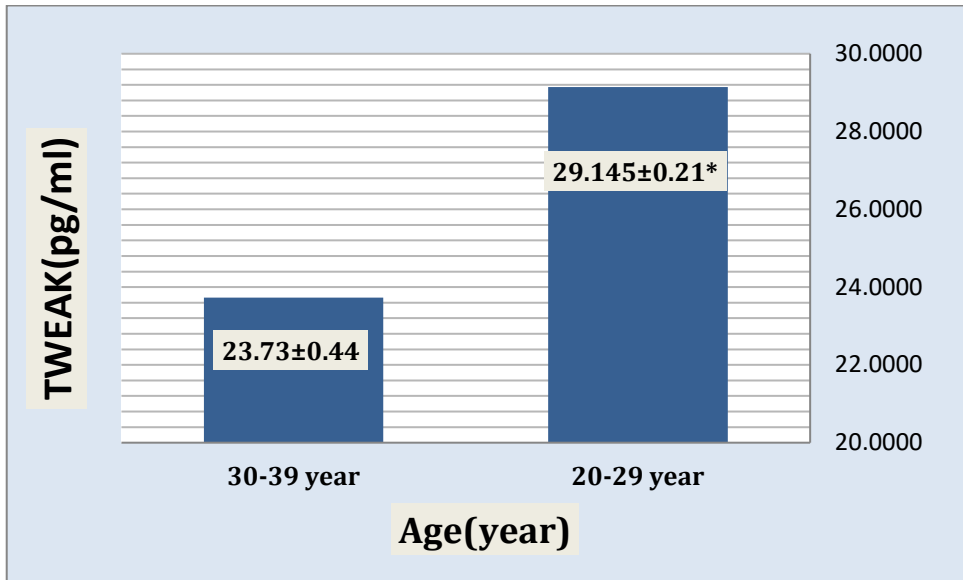
**Fig. 3.** TWEAK level in Hb groups less than 11.7 and more than 11.7 of patients with systemic lupus erythematosus.

No significant differences (P-Value > 0.05)

Hb < 11.7 n=28, Hb > 11.7 n=32

### 3.4 Comparison of biomarker in systemic lupus erythematosus according to ages

Figure (4) explains the results of serum TWEAK level in all age groups of systemic lupus erythematosus, these results mentioned significant increase ( $p < 0.05$ ) in TWEAK level of age group (20-29) year about (29.145±0.21) pg/ml comparison with that age group (30-39) (23.73±0.44) pg/ml.



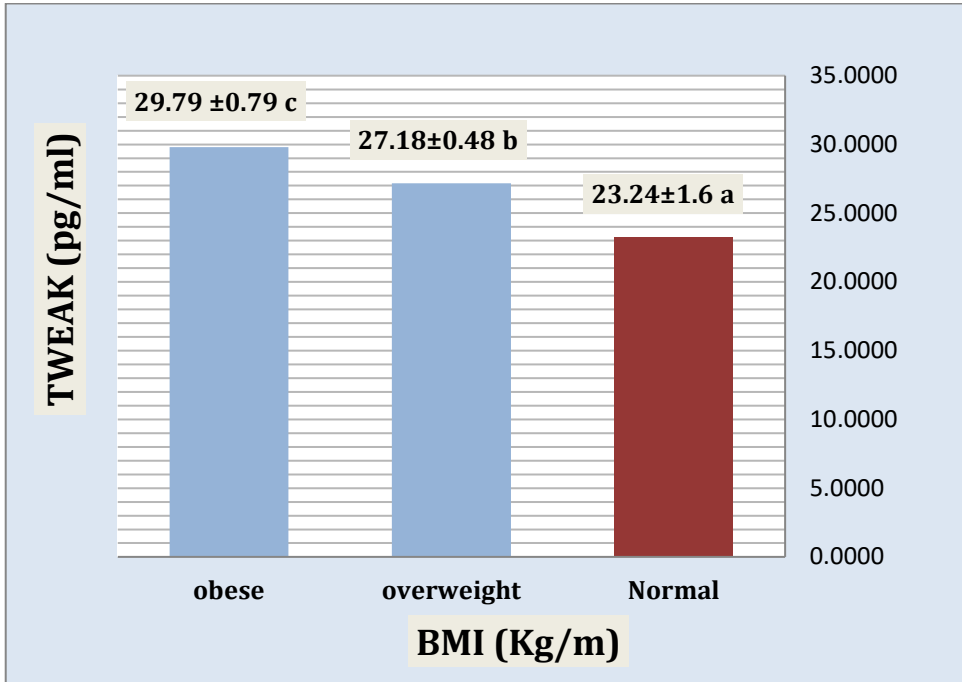
**Fig. 4.** TWEAK level (pg/ml) in both age groups of patients with systemic lupus erythematosus.

\* refer to significant differences (P-Value < 0.05)

age 20-29 n=35, age 30-39 n=25

### 3.5 Comparison of biomarker in systemic lupus erythematosus patients according to BMI

Serum TWEAK level in three groups of patient as shown in figure (5). The results indicate the presence of a significant decrease ( $p < 0.05$ ) in serum TWEAK level of normal weight groups ( $23.24 \pm 1.6$ ) pg/ml, in comparison with overweight and obese group ( $27.18 \pm 0.48$ ) pg/ml and ( $29.79 \pm 0.79$ ) pg/ml respectively.



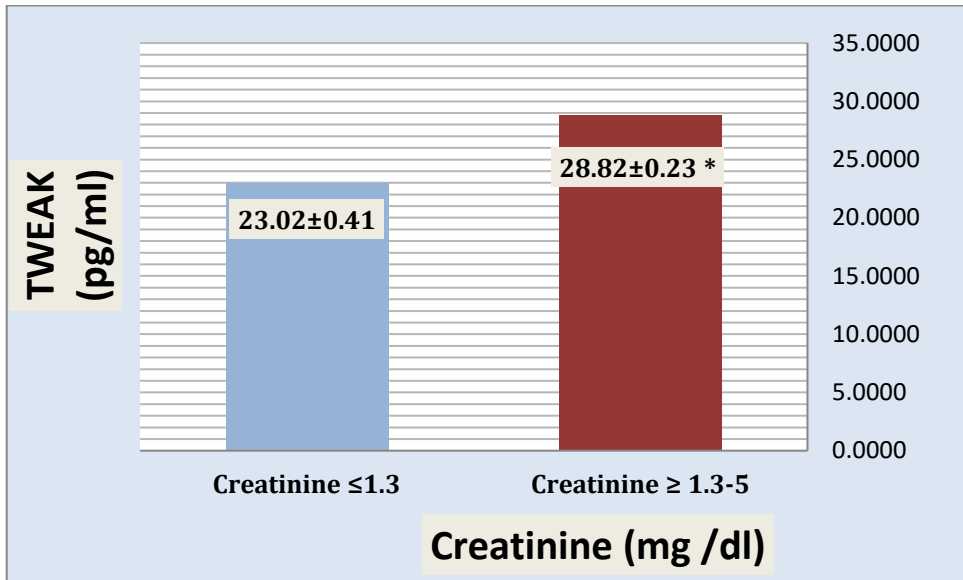
**Fig. 5.** TWEAK level (pg/ml) in normal weight, overweight and obese groups of patients with systemic lupus erythematosus.

Different letter refer to significant differences (P-Value < 0.05).

Normal weight n=20 overweight n=15 obese n=25

### 3.6 Comparison between biomarkers in systemic lupus erythematosus patients according to creatinine

Figure (6) the statistical analysis revealed to significant increase ( $p < 0.05$ ) in serum TWEAK levels of creatinine  $\geq 1.3$ -5 group ( $28.82 \pm 0.23$ ) pg/ml as compared to the the creatinine  $\leq 1.3$  group ( $0.867 \pm 0.090$ ) pg/ml of systemic lupus erythematosus patients.



**Fig. 6.** TWEAK (pg/ml) level in creatinine  $\leq 1.3$  and creatinine  $\geq 1.3-5$  groups of systemic lupus erythematosus patients.

\* refer to significant differences (P-Value  $< 0.05$ ).

Creatinine  $\leq 1.3$  n=20 creatinine  $\geq 1.3-5$  n=40

## 4 Discussion

Fig. 1. showed a significant increase in creatinine concentrations of systemic lupus erythematosus patients in comparison with control group. Recent study Ibrahim *et al.*, (2020), has been estimated that Lupus nephritis considers the main disease that may be occurred as a result of action SLE, the facilities of cell adhesion molecules (CAM) include adherence of leukocyte and control their movement into inflame tissues [17]. study of Rani *et al.*, (2021) who reported that Lupus nephritis (LN) develops as a result of immunological abnormalities [18]. Lupus nephritis is the disease of the kidneys due to the deposition of autoantibodies that cause inflammation. About 30–50% of lupus patients will develop LN within the first 6 months to 3 years of being diagnosed with SLE [19]. The results of figure Fig. 2. revealed significant significant increase in level of TWEAK in patients with systemic lupus erythematosus in comparison with that of control group. The current study agree with study of Chen *et al.*,(2019) that reported serum TWEAK levels are higher in patients with SLE or subacute cutaneous lupus erythematosus than patients with discoid lupus erythematosus or healthy controls, (TWEAK) is a proinflammatory cytokine participating in the pathogenesis of systemic lupus erythematosus (SLE) [20]. Another study has been shown that increased TWEAK levels due to inflammation stimulate the release of cytokines such as TNF-  $\alpha$ , IL-1, IL-6, granulocyte-colony stimulating factor (G-CSF), and interferon- $\gamma$  monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1 alpha (MIP-1 $\alpha$ ), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) [21]. The results of Fig. 3. referred a no significant difference in TWEAK level between group of Hb less than 11.7 and group of Hb more than 11.7 of systemic lupus erythematosus patients. Another study has been suggested that TWEAK-triggered NF- $\kappa$ B can induce caspase expression while caspase signaling mediates the effects of IFN- $\gamma$ , which forms a circuit to inhibit hematopoiesis [22]. The result of Fig.



4. refer to significant increase in TWEAK level of age group (20-29) year comparison with that age group (30-39) of systemic lupus erythematosus patients. The explain may be the significant increase in TWEAK and S100A8 in age (20-29)year may be discuss as a level of its marker appear in low degree of inflammation at earlier stage all biomarkers activate cellular immunity –T-lymphocytes and release of different cytokines also infiltration and migration anumber of leukocyte such as monocytes, macrophage and neutrophils and these associated with disease activity at ages(20-29)years. The results of Fig. 5 The results indicate the presence of significant decrease in serum TWEAK level of normal weight groups in comparison with overweight and obese group. A study of Tiller *et al.*, (2009) also has been shown that that TWEAK can interfere with the differentiation ability of several cell types, including myogenic, osteoblast, chondrocyte, and erythroblast lineages. In addition, TWEAK can also inhibit adipocyte differentiation at an early stage, as indicated by a rapid reduction of the key adipogenic transcription factors Peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) and CCAAT/Enhancer Binding Protein  $\alpha$  (C/EBP $\alpha$ ) [23]. The result of Fig. 6 showed significant increase in serum TWEAK levels of creatinine  $\geq 1.3$ -5 group group as compared to the the creatinine  $\leq 1.3$  group of systemic lupus erythematosus patients . The result of Current study agree with study of Xue *et al.*, (2017) that documented LN patients had significantly higher TWEAK expression in glomeruli and tubulointerstitium compared with normal controls, TWEAK regulated renal damage, and this was associated with activation of the type I IFN pathway [24, 25, 26].

## 5 Conclusion

The current study concluded that TWEAK was a prognostic marker for detection of systemic lupus erythematosus with renal disease (LN) associated with some complication.

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