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Relationship between Toll-like receptors and pathogenesis of Systemic Lupus Erythematosus

Basma Ahmed Ratib[1], Asmaa Mohammed Saud[1]

1 Institute of genetic engineering and biotechnology for post graduate studies , University of Baghdad;basima @ige.uobaghdad.edu.iq. 0000-0003-4952-8087

2 Institute of genetic engineering and biotechnology for post graduate studies , University of Baghdad;mayada.h@ige.uobaghdad.edu.iq.

* Correspondence: basima @ige.uobaghdad.edu.iq; Tel.: 009647831533300

Abstract: Systemic Lupus Erythematosus is a chronic inflammatory disease characterized by a loss of self-antigen tolerance and the development of high titers of serum autoantibodies, with a wide range of clinical manifestations and complex etiologies. Its etiology is influenced by a variety of genetic, hormonal, immunologic, and environmental factors. SLE affects around 90% of women of reproductive age. The study's aim is to evaluate the serum levels of Toll-like receptor 7 (TLR7) in a sample of Iraqi SLE patients and its potential relationship with other clinical and laboratory parameters. The study included 100 female patients and 50 healthy females with an age range of (16-65) years old and healthy individuals with an age range of (16-65) years old, and mean ages of (35.72 ±11.66 and 35.72 ±11.66) respectively. The current study is performed to estimate the serum levels of (TLR7, ANA, Urea, creatinine, and Vitamin D3) and a laboratory investigation for ESR, hemoglobin, and white blood cells by using the enzyme-linked immunosorbent assay (ELISA)and automated Fujifilm. Serum levels of TLR7 were increased in the SLE patients compared to control, and a significant difference has been observed (P≤0.01) among SLE patients as compared to control. Urea, creatinine, and ESR were significantly higher, at the same time the Vitamin D3, hemoglobin, and white blood cells were significantly lower (p< 0.01) among SLE patients as compared to control. On the other hand, there was no evidence for any correlation between TLR7 serum level and disease laboratory investigation.

Keywords: Anti-nuclear autoantibody, Haemoglobin, Interferon, SLE, Toll-Like Receptor-7, WBC.

1. Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by multi-systemic and multi-organ involvement, The presence of numerous autoantibodies that induce the development and deposition of immune complexes as well as other immunological processes, is related to the symptoms of SLE [1]. The mechanisms underlying this disease are complicated and unknown, but they are thought to be linked to environmental activation of the body's immunological dysfunction, and defective immune regulation [2]. Antinuclear antibody (ANA), anti-double-stranded DNA antibodies, complement 3 protein (C3), complement 4 protein (C4), and leukopenia are among the few indicators that help physicians make decisions on disease diagnosis and prognosis [3]. Human plasmacytoid dendritic cells (pDCs) are thought to be important contributors to the development of autoimmune disorders, in SLE they can produce cytokines and chemokines in response to autoimmune complexes such as self-DNA and small nuclear ribonucleoprotein particles (snRNPs). TLR7 had a higher degree of expression on human pDCs than the other TLRs. Continuous stimulation of pDCs by endogenous nucleic acids activates the type I IFN system in SLE, resulting in prolonged type I IFN production. TLR7 and TLR9 are both associated with B cell activation, and IFN- production in SLE [4,5]. B cell abnormalities play a key role in the development of systemic lupus erythematosus (SLE) [6]. Both central and peripheral tolerance mechanisms inhibit the growth and survival of B lymphocytes that react to self-antigens [7]. When these tolerance mechanisms fail, autoreactive B cells are produced, which contribute to the pathogenesis of SLE. TLRs, which sense nucleic acids in endosomes, are responsible for the cell-intrinsic loss of B cell tolerance to autoantigens in people with SLE. TLR7 drives the extrafollicular B cell response and the germinal center reaction, which are both involved in autoantibody production and disease pathogenesis. Autoreactive B cells identify DNA-associated antigens (such as unmethylated cytosine-phosphate-guanosine (CpG) motifs) and RNA-associated antigens (such as the single-stranded RNA (ssRNA) short nuclear RNA, U11) in systemic lupus erythematosus (SLE). These self-antigens are thought to be released as a result of dysregulated pathways that

cause an increase in neutrophil extracellular traps (NETs), necrotic cells, or apoptotic cells [8] .The B cell receptor (BCR) recognizes these autoantigens on the surface of B cells and triggers their cellular internalization . Once within the endosomes, In B lymphocytes, self-antigens can activate Toll-like receptor 7 (TLR7) and/or TLR9. TLR7 expression is greater in women than in men because TLR7 is located on X chromosome [9,10], The TLR7 gene escapes X-chromosome inactivation, resulting in considerably higher amounts of TLR7 protein than in male cells [11]. Genetic or environmental factors that contribute to TLR7 overexpression enhance an individual's sensitivity to SLE (such as gender, nutrition, and the cytokine media, especially the amount of type I interferons) [8]. In physiological circumstances, TLR9 inhibits TLR7 signaling, and this chemical protects people from developing SLE. TLR9 function disruption, on the other hand, may favor TLR7 signaling and contribute to the development of SLE [8]. The aim of this study is to assess the relationship between serum levels of TLR7 and clinical and laboratory indicators in Iraqi female patients with SLE.

2. Materials and Methods

Subject

After obtaining the approval of the Ethics Committee in the Biotechnology Department, College of the Science , University of Baghdad. The peripheral blood samples were collected from all participants of 100SLE patients. Informed consent was obtained from all participants before their inclusion in the study. All SLE patients met the classification criteria of the American College of Rheumatology [12].

Inclusion and Exclusion criteria

We used available data to apply typical inclusion criteria for SLE, including the age of (16-65) years and a positive diagnosis of SLE EULAR/ACR [12]. Patients with other immunological disorders or overlapping autoimmune diseases, as well as patients with juvenile SLE (early-onset) who were less than 16 years old, were excluded from the study.

Laboratory Investigations

The laboratory tests were conducted to evaluate the immunological and routine tests. The patients and controls were tested for total serum levels of TLR-7 as well as anti-nuclear autoantibody (ANA), complete blood count (CBC), Erythrocyte sedimentation rate (ESR), Vitamin D3 (25(OH)D), blood urea and creatinine levels. Blood (3 ml) was withdrawn from each subject (patients and controls), divided into sterile gel tubes, and left for about two hours to clot. The sample was then centrifuged at 3000 rpm for 15 minutes to separate the serum which was stored at -20°C until assayed. ELISA kits were employed to assess levels of total TLR-7(BioSource Inc, British) and ANA [13]. The blood urea and creatinine levels were measured using an automated Fujifilm. Two milliliters of venous blood were withdrawn from each subject by vein-puncture under an aseptic technique by multi-sample syringe. For estimating complete blood count, whole blood was placed in EDTA tubes and tested by a Hematology analyzer.

Toll-Like Receptor-7 Measurement

Serum level of TLR-7 titer was measured by using ELISA technique, commitment to the instructional manual provided by manufacturer BioSource Inc, British (Cat. No RDEEH2015).

Statistical analysis

To determine the effect of different components in research parameters, SPSS software (Statistical Package for the Social Sciences) version-13 was used to analyze the subjects' data. Parametric results (quantitative) were used to compute TLR7 levels, whereas non-parametric data (qualitative) was calculated using means and standard deviation. Additionally, for comparisons, the Pearson Chi-square test was utilized, and Spearman correlation was used to test the correlation between different study parameters. If the P-value is considered significant if it is less than 0.05 [14].

3. Results

The number of the Systemic Lupus Erythematosus patients selected for the current study was in total, 100 of SLE patients and 50 healthy control individuals, all participants in this study were female. The age range of SLE patients was between 16 to 65 years with a mean and standard deviation value equal to (35.72 ± 11.66 years). The age range of the controls was identical to the age range of patients (34.05 ± 11.02years). The participants were divided into three age groups; 16-29, 30-49 and > 50 years. The percentage of the SLE patient in the same age groups was (36.00%, 54.00%, and 10.00%), respectively. While the percentage of the healthy subjects for the same age groups was (42.00%, 48.00%, and 10.00%), respectively. as shown in Table1.

Table 1. Distribution of subjects according to age, and age group.

Groups		Patients (No.=100)	Controls (No.=50)	*p*-value
Age (mean±S. D.) year		35.72 ±11.66	34.05 ±11.02	0.491
Age Group (No.%)	16-29 years	36(36.00%)	21(42.00%)	0.382
	30-50 years	54(54.00%)	24(48.00%)	0.379
	<50years	10(10.00%)	5(10.00%)	1.00

p-value: probability; No.; Number, S. D.; standard deviation

One of the essential parameters in SLE diagnosis is the serum level of total TLR-7. Table 3 shows the average concentration level of total TLR-7 in the serum of SLE patients in comparison with that of the control. The total TLR-7 mean level of the systemic lupus group (4.68 ±1.65) was higher than that of the control group (1.86 ±0.41), with a highly significant difference (P=0.0001), as shown in Table 2.

Table 2. TLR7 level of systemic lupus erythematosus patients in comparison with controls.

Groups / (No,)	TLR7 level mean ± SD (ng/ml)	*p*-value
Patients / 100	4.68 ±1.65	0.0001
Control / 50	1.86 ±0.41	

p-value: probability; No.; Number of subjects, S. D.; standard deviation.

All the laboratory tests of the female SLE patients and healthy control enrolled in this study were described in Table 3. There was a significant difference in Urea, creatinine, ANA and ESR were significantly higher, at the same time the Vitamin D3, hemoglobin, and white blood cells were significantly lower among SLE patients as compared to control

Table 3- Laboratory investigations in all studied group.

Parameters Mean ±SD or N (%)	Patients (N=100)	Controls (N=50)	*P*-value
ESR (mm/hr.)	27.12 ±15.27	15.40 ±5.66	0.0001
WBC (10^3/mL)	4.21 ±2.04	7.61 ±1.72	0.0001
HB (g/dl)	10.48 ±2.35	13.01 ±1.67	0.0001
V.D 3	24.78 ±9.21	37.53 ±5.93	0.0001
Urea (mg/dl)	51.58 ±25.94	29.12 ±7.82	0.0001
Creatinine (mg/dl)	1.906 ±1.76	0.720 ±0.17	0.0001
ANA positivity	7.15 ±3.34	0.296 ±0.25	0.0001

Values are expressed as N: number in each parameter, %: percentage in each group and mean ± SD. The abbreviations are; WBC: white blood cell, Hb: hemoglobin, S: serum, V.D3: vitamin D3, ANA: antinuclear antibody. Normal ranges: WBCs (10^9/L), 4.00-10.00; HB (g\dl), 150-450; urea (mg\dl), 15-45; Creatinine (mg\dl), 0.3-0.7, V.D3: (ng/ml), 30-50. *Correlation is significant at the 0.05 level (2-tailed)

Personal correlation between TLR-7 levels and laboratory parameters including ANA, WBC, Hb, ESR, urea, creatinine, and Vit.D3, statistically show an insignificant correlation with TLR7 according to laboratory examinations, as shown in Table 4.

Table 4: Correlations between TLR-7 and laboratory parameters.

Parameters	TLR-7 in SLE patients (N=100)	
	R	**P-value**
ANA	-0.003	0.985 NS
WBC	-0.12	0.376 NS
Hb	-0.14	0.339 NS
ESR	0.04	0.769 NS
Urea	-0.21	0.140 NS
Creatinine	-0.14	0.338 NS
Vit. D3	0.12	0.396 NS

*. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed). R: Pearson's correlation.

4. Discussion

Systemic lupus erythematosus is a multisystemic autoimmune disease with a wide range of clinical manifestations and a high risk of morbidity and mortality [15]. SLE is characterized by an increase in the production of autoantibodies directed against self-antigens, as well as a wide range of clinical symptoms and a flare-up course that is completely unpredictable [16]. Due to the low prevalence of the disease in the male's, all the studied group were females, due to different hormonal secretions between males and females, SLE primarily affects women in their reproductive years, and affect any organ in the body [17], estrogen and prolactin (sex hormones associated with female reproduction and development) higher levels of these hormones can allow more B cells to circulate in the body. Next, the increased level of B cells can lead to an immune response, and may trigger symptoms of SLE, this can be attributed to a risk factor for female gender and age in the genesis of SLE. With respect to age, SLE is thought to be a disease that affects middle-aged individuals more than younger or older persons. The recorded age of this study ranged from 16 to 65 years with a mean age of (35.72years). These results corresponds with [18] study that records the peak range of SLE Iraqi patients to be between <20 to >50. Current study showed that the thirtieth decade is regarded as a critical risk factor, this is in agreement with [19] who showed that The most susceptible age for SLE is 20–39 years for females. Women were more frequently affected than men for every age and ethnic group [20].The current study applied to females of different ages SLE cases, most studies agree that females are affected at considerably higher rates than males [21]. Sex hormones affect immunological responses and modulate molecular pathways in the innate and adaptive immune systems [22]. Women's higher rates of SLE may be due to endogenous estrogen, X chromosomal inactivation failures, increased Toll-like receptor gene products, and alterations in microRNA activity, among other factors [23].

Human SLE has been associated with TLR7 overexpression [24]. in the current study there was a significant difference in mean TLR7 serum levels between SLE patients (4.68 ±1.65) and healthy controls (1.86 ±0.41), so (P= 0.0001). This is because TLR7 produces type I interferon and promotes the activation of autoreactive B lymphocytes [25]. The results of this study showed a significant increase in the serum level of TLR7 in SLE patients, this result was supported by [26] who showed that total TLR-7 (p<0.01) levels were significantly increased in lupus patients compared to age-matched controls.

Antinuclear antibodies (ANA) more specific autoantibody testing may be helpful in the diagnosis of suspected SLE or ANA-associated disease [27]. Also, it is discussed as an entrance criterion for SLE, while the number of ANA-negative patients worldwide is small (<5%) [28]. Evidence suggests that ANA responses can deteriorate over time as a result of disease progression or medication side effects [29].The current study finds that ANA positivity of 98% in SLE patients, as a result, shows a significant difference in the mean of ANA serum levels between SLE patients and healthy controls, however these result correspond with [30] study on Iraqi SLE patients who recorded ANA test:98%. Also, ANA shows an insignificant correlation with TLR7. This is in disagreement with [31] who reported that TLR7 ligand stimulation in SLE patients resulted in anti-nuclear autoantibodies (ANA) production.

Evidence suggested that Vitamin D3 could exert some of its immunomodulatory effects in SLE patients by affecting the expression levels of some TLRs [32]. Vitamin D3 in the current study shows an insignificant correlation with TLR7. In contrast, it offers a significant difference in the mean of V.D3 serum levels between SLE patients and controls (P value=0.0001).This is in agreement with Meza-Meza *et al* they reported that Vitamin D deficiency is more common in people with systemic lupus erythematosus (SLE) than in healthy people [33].

The laboratory investigations in total (urea, creatinine, ESR, hemoglobin, and WBC) show an insignificant correlation with TLR7. Studies reported that TLRs, which are found on both leukocytes and resident renal cells, play a role in the start of glomerulonephritis and the progression of kidney injury by bridging the gap between innate and adaptive immune responses [34,35]. Other studies found a link between B lymphocyte infiltration in the kidneys and glomerulonephritis, greater activity and chronicity indices, and higher serum creatinine levels, highlighting the prognostic relevance of tubulointerstitial involvement [36].

5. Conclusions

The results suggested that female gender and age play a role in the etiology of SLE. Increasing TLR7 serum level in patients compared to control indicate that TLR7 is an indicator of the onset of the disease. There was no evidence of a correlation between TLR7 serum levels and any clinical signs or (laboratory investigations) in this study.

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