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Article

The Role of CX3CL1-CX3CR1 Axis, C3, C4 & ESR Abs in pathogenicity of Iraqi patients with SLE

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ABSTRACT

Background: Systematic Lupus erythematosus (SLE) has been described as a chronic inflammatory illness where chemokines play an essential role in its pathogenesis CX3CL1and CX3CR1 are chemokines that described their crucial role in immune response in SLE patients. Aim of the study: To evaluate the serum level of CX3CL1- CX3CR1, C3, C4 & ESR Abs in SLE patients without treatment and compare their level with those under treatment (hydroxychloroquine,predeslone 5-20mg, D3). Patients collecting and Methods: 120 females with SLE and healthy, with ages ranging between 20-40 years, were involved in this investigation from Medical City (Consultant of Arthritis, Consultant of Dermatology, Lobby of Hematology and Arthritis)/ Baghdad Teaching Hospital and from Al-Imameen Al-Kazimeen Teaching Hospital from August 26 to October 18, 2021. The samples included 80females with SLE (40 females as early diagnosed patients (G2) without treatment, 40 females as patients that received treatment subjects (hydroxychloroquine, predeslone 5-20mg, D3) (G3), while the control group included 40 healthy females (G1). Five mL of venous blood were obtained from patients and healthy females for measuring C3, C4, ESR and serum levels of CX3CL1 and CX3CR1, which were measured using the ELISA method. Results: Our findings demonstrated a significant increase in the serum levels of CX3CL1, CX3CR1, and ESR. Also, there were significant decreases in serum levels of C3 and C4 in SLE patients (with and without treatment) compared to the control group, and a significant difference was detected between SLE patients without treatment and patients receiving treatment. Conclusion: Based on our results, CX3CL1 and CX3CR1 chemokines may have a role in the pathogenesis of SLE as they are increased in SLE patients. In addition, serum CX3CL1 levels can be used as an independent biomarker of SLE activity. Furthermore, low levels of c3 and c4 and high levels of ESR are considered diagnostic indicators of SLE disease in people.

Keywords: *Systemic lupus erythematosus, CX3CL1; CX3CR1. C3,C4*

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by autoantibody production and multisystem inflammation[1-3]. It is also considered a systemic immune disease that affects more than one organ if it occurs. Studies in humans and experimental animal models have revealed a complex interaction of genetic and environmental factors in SLE, leading to immune dysregulation and immunological tolerance breakdown that, in turn, results in autoantibody production and multi-organ inflammation[1,2]. Systemic lupus erythematosus usually develops between adolescence and adults, ages 15-44, and primarily affects females of reproductive age. Many Factors contributing to the activation of SLE. This disease results from a defect in the immune system consisting of autoantibodies that can bind to autoantigen attack parts of the body[4]. The disease arises due to an abnormal response to environmental factors (such as exposure to ultraviolet rays contained in sunlight and light rays emitted by artificial lights, after exposure to infections or taking certain medications) in people with a genetic predisposition. As a result, they attack body tissues, causing inflammation or damage; the response to it, s-shaped by the formation of autoantibodies, immune complexes are deposited in blood vessels and body organs and increased risk of lupus affected by hormones, estrogen- or prolactin, psychosocial factors[5]. It is also associated with the use of oral contraceptives at an early age at menopause[6]. The symptoms and signs of the disease are numerous because it affects multiple systems in the body and varies from one case to another. The predominant symptoms are joint pain and inflammation (Arthralgia and Arthritis)[7], a rash on the face and skin (malar and other skin rashes), inflammation of the membranes around the lungs and heart (pleuritis or pericarditis), infection also involving the kidneys and central nervous system (renal and CNS involvement[8,9]. Chemokines are cytokines with chemical activity. (Greek -kinos, movement) are a family of small cytokines or signaling proteins secreted by cells. Their name is derived from their ability to induce directed chemotaxis in nearby responsive cells[10]. Cytokine proteins are classified as chemokines according to behavior and structural characteristics. In addition to being known for mediating chemotaxis, chemokines are all approximately 8-10 kilodaltons in mass and have four cysteine residues in conserved locations that are key to forming their 3-dimensional shape[11]. Chemokines are found in all vertebrates, some viruses and some bacteria, but none have been found in other invertebrates[12,13]. CX3CL1 is a large cellular protein comprising 373 amino acids, contains multiple domains and is the only known member of the CX3C chemical family. It is also known as fractalkine (in humans) and neurotactin in mice[14]. In contrast, cell-associated chemokines promote strong adhesion of leukocytes to activated endothelial cells, where they are constitutively expressed. CX3CL1 accentuates its adhesive and migratory functions by interacting with the CX3CR1 chemokine receptor[15]. Its gene is located on human chromosome 16 and some CC chemokines known as CCL17 and CCL22. Fractalkine is commonly found throughout the brain, particularly in neurons, and its receptor is known to be present in microglia[2]. It was also found to be essential for the migration of microglia. CX3CL1 is also upregulated in the hippocampus within a short time after spatial learning. It also plays a role in cancer diseases[3]. CX3CR1 chemoreceptor 1 (CX3CR1), also known as fractalkin receptor or protein-coupled receptor 13 (GPR13), is a protein that in humans is encoded by the CX3CR1 gene[7]. Expression of this receptor appears to be associated with lymphocytes. Monocytes also express CX3CR1 and plays a crucial role in monocyte survival[10]. Moreover, the Role of CX3CL1 and its receptor CX3CR1 in SLE Through the study, it was shown that CX3CL1 caused an increase in pain, while the injection of anti-CX3CR1 antibodies reduced the occurrence of pain. From this, it is

concluded that CX3CL1 and its receptor CX3CR1 represent a new and promising target for treating pain during disease[6]. Complement is a mediator of inflammation, and complement deficiency predisposes to the development of SLE. Inherited complement C4 deficiency, whether partial or complete, confers a high risk of developing SLE, whereas C3 deficiency is only rarely associated with SLE-like illness; the association between complement .deficiencies and SLE supports an important role of complement in preventing immune complex-mediated tissue damage. However, increased activation of complement without deficiencies may also contribute to the tissue damage in SLE[9].

MATERIALS AND METHODS

This study included 120 females (with SLE and healthy) with ages ranging between 20-40 years, which were collected from Medical City (Consultant of Arthritis, Consultant of Dermatology, Lobby of Hematology and Arthritis)/ Baghdad Teaching Hospital and Al-Imameen Al-Kazimeen Teaching Hospital from August 26 to October 18, 2021. The samples were included in the control group of 40 healthy females (G1). 80 females with SLE, 40 females as early diagnosed patients (G2) without treatment, and 40 females as patients that received treatment subjects (G3); all samples were collected and diagnosed by the consultant in the unit in the hospitals according to the protocols followed by them through the official specialist. Data related to age, duration of illness (as initial diagnosis or exposure to treatment), and exposure to other diseases were recorded. c3,c4 and erythrocyte sedimentation rate (ESR) were assessed, in addition to the fact that clinical examination by the consultant doctors made the diagnosis. The control and patient groups collected the venous blood (10 mL). The blood samples were distributed in test tubes, and the serum was separated using a centrifuge (3000 rpm for 30 min) and then transferred into 0.5 mL Eppendorf tubes that were immediately frozen at -20º C until used in the determination of studied parameters. CX3CL1 and their receptors CX3CR1 were measured in all under-studying groups using the ELISA technique. C3, C4 analysis was performed using (Cobas 6000) device, and the results are presented in a g/L, as well as determining the degree of functional impairment of the organs and clinical signs of the disease.

RESULTS

This study included 40 healthy females(G1) and 80 Iraqi females with SLE, which were divided into two groups; the first included 40 patients with early diagnostic (G2), while the second group included 40 treated patients (G3). The understudied group age ranged from 20-40 years, the result of age (Mean+S.E) for G1, G2, and G3 was (28.7±0.74, 29.76±0.91, 31.7±0.85) years, as shown in Table 1.

Groups	Control	Early diagnostic	Treated
	Mean+S.E		
Age	28.7±0.74	29.76±0.91	31.7±0.85

Table 1. The (Mean±S.E) of age for all understudied groups

Table 2 illustrates that the serum level of CX3CL1 significantly increased (P<0.001) in both SLE patients (G2 and G3) in comparison to the control group. Furthermore, there was a significant difference between G2 and G3, where a significant decreasing level was shown in G3 compared to G2, that is, an improvement in CX3CL1 levels in the patients taking the treatments. The means of CX3CL1 were (69.74±6.50), (298.72±18.23) and (133.74±11.38) pg/mL in G1, G2 and G3, respectively.
Additionally, it was found that the serum levels of CX3CR1 significantly elevated (P<0.005) in SLE early diagnosed patients but not treated SLE patients as compared to the control group. On the other hand, significant differences were revealed between G2 and G3 groups, where it was decreased in SLE-treated patients. The mean of CX3CR1 was (107.09±5.55) in G1, (399.89±28.73) in G2 and (122.57±6.61) pg/mL in G3.

Parameter	Group	Mean+S.E	P value		
			Control	Early diagnostic	Treated
CX3CL1	Control	69.74±6.50	-	0.001	0.001
	Early diagnostic patients	298.72±18.23	0.001	-	0.001
	Treated patients	133.74±11.38	0.001	0.001	-
CX3CR1	Control	107.09±5.55	-	0.001	0.53NS
	Early diagnostic patients	399.89±28.73	0.001	-	0.001
	Treated patients	122.57±6.61	0.53NS	0.001	-

Table 2. Serum level of CX3CL1 - CX3CR1 in patients & control

On the other hand, as shown in Table (3), the serum levels of C3 and C4 significantly decreased among the understudied groups of patients compared to healthy individuals. The mean of C3 was (1.39±0.03) g/L in G1, (0.67±0.02) g/L in G2 and (1.09 ± 0.05) g/L in G3 group. Whereas the means of C4 were (0.82±0.04), (0.13 ± 0.03) and (0.55 ± 0.05) g/L in G1, G2 and G3 groups, respectively. Furthermore, there was a significant difference between G2 and G3.
Moreover, The result of ESR was demonstrated in Table (3), and the statistical analysis found a significant elevation (P<0.001) in ESR among the studied groups as compared to the control group. The mean of ESR in G1 was (13.19 ± 0.53), (79.32 ± 1.87) in G2 and was (42.85±2.38)]mm/h in G3. Also, there was a significant difference was recorded between G2 and G3.

Parameter	Group	Mean+S.E	P value		
			Control	Early diagnostic	Treated
C3	Control	1.39±0.03	-	0.001	0.001
	Early diagnostic patients	0.67±0.02	0.001	-	0.001
	Treated patients	1.09±0.05	0.001	0.001	-
C4	Control	0.82±0.04	-	0.001	0.001
	Early diagnostic patients	0.13±0.03	0.001	-	0.001
	Treated patients	0.55±0.05	0.001	0.001	-
ESR	Control	13.19±0.53	-	0.001	0.001
	Early diagnostic patients	79.32±1.87	0.001	-	0.001
	Treated patients	42.85±2.38	0.001	0.001	-

Table 3. Serum level of C3,C4 and ESR in patients and control

As shown in Table 4. The statistical analysis of CX3CL1 levels founded a significant difference (P<0.01) in serum of SLE patients (G2) of age groups (20-30) and (30-40) years while no significant difference in (G3) Additionally, there was significant difference (P<0.034) in (CX3CR1) levels between (20-30) and (30-40) years in (G2), but not in (G3).

.Moreover, The results showed a significant difference (P<0.017) in ESR between (20-30) and (30-40) years age groups in (G2), while there is no significant difference in (G3).

.On the other hand, levels of C3 and C4 in the serum of SLE patients according to the age groups (20-30) and (30-40) years, the results recorded a highly significant difference (P<0.001) in C3 levels in age groups (20-30) and (30-40) years in (G2), but not with (G3) group. While no significant difference in C4 levels in all understudied age groups

DISCUSSION

Our findings revealed that serum CX3CL1 and CX3CR1 levels in early diagnosed and treated SLE patients were considerably higher than in control and significantly different among SLE patients, especially after the treatment. These results were consistent with the previous studies [11,12] that reported that serum Fkn was significantly higher in SLE patients than healthy individuals. In addition, Fkn levels were significantly higher in all children with SLE when compared with healthy children[3]. Moreover, [13] who referred to increasing of CX3CR1 in patients with SLE.

Parameter	Groups (yrs.)	Early diagnostic (Mean+S.E)	P value	Treated group (Mean+S.E)		P value
CX3CL1	20-30	252.34±25.05	0.01	117.72	19.44	0.25 NS
	31-40	345.09±24.02		144.42	13.78	
CX3CR1	20-30	339.43±38.76	0.034	115.89	12.53	0.41 NS
	31-40	460.34±40.04		127.02	7.26	
ESR	20-30	74.90±2.69	0.017	41.29	4.09	0.6NS
	31-40	83.73±2.38		43.88	2.92	
C3	20-30	0.75±0.03	0.001	0.98	0.06	0.05
	31-40	0.59±0.03		1.17	0.07	
C4	20-30	0.11±0.03	0.548NS	0.47	0.08	0.19NS
	31-40	0.15±0.04		0.61	0.06	

Table 4. Correlation of age and studied parameters

The complement components C3 and C4 are important units that stimulate the complement pathways of the immune system. This study found that the serum levels of C3 and C4 significantly decreased among the understudied groups of patients compared to healthy individuals. The current finding agrees with the previous studies (Liu et al., 2018). Furthermore, The low levels of C3 and C4 in the sera of SLE patients means that the patients have increasing autoantibodies and vice versa [14].

The statistical analysis recorded a significant elevation in ESR among the studied groups compared to the control group. The result is in agreement with the previous studies. Fifteen found a slight increase in ESR. In addition, the elevation of ESR increases with the severity and progression of the disease [4].

CONCLUSION

Based on our results, it can be concluded that CX3CL1 and CX3CR1 chemokines may have a role in the pathogenesis of SLE as they are increased in SLE patients. In addition, serum CX3CL1 levels can be used as an independent biomarker of SLE activity. Furthermore, low levels of c3 and c4 and high levels of ESR are considered diagnostic indicators of SLE disease in people.

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