University of Thi-Qar Journal of Science (UTJsci)
E-ISSN: 2709-0256, P-ISSN: 1991-8690, Vol. 11, No. 2, dec. 2024

Estimation of TNF- α and some immunological parameters in patient with systemic lupus erythematosus

Sarah Hashim Kazem AL-Abdullah[1a*] and Murtada Hafedh Hussein[1b]

[1]*Dept. of Pathological Analysis, College of Science, University of Thi-Qar, Nasiriyah, Iraq.*
[b]E-mail: dr-murtadahafedh@sci.utq.edu.iq
[a*]Corresponding author: sara.kadhem@sci.utq.edu.iq

Received:2024-03-13, Revised: 2024-03-19, Accepted: 2024- 0 4-29, Published: 2024-12-05

***Abstract*— Systemic Lupus Erythematosus (SLE) is a multifactorial chronic systemic autoimmune disease. It is characterized by a lack of immune tolerance to autoantigens such as nuclear antigens. In this study, 25 individuals diagnosed with SLE had their blood samples drawn, while 25 healthy individuals were used as a control group. The results of this investigation show that the patients' TNF-α levels (106.9 ± 10.8) significantly increased more than 52.86 in the control group. Comparing the immunological parameters of the patients to the healthy group, the study revealed a decrease in the eosinophile, basophile, lymphocyte, and monocyte counts (010 ± 0.003, 0.022 ± 0.004, 1.71 ± 0.66, 0.21 ± 0.047 respectively) and a rise in the neutrophile and immature granulocyte counts (7.43 ± 1.66, 2.68 ± 0.46 respectively) in SLE patient.**

***Keywords*— SLE, TNF α, immunological parameters**

I. INTRODUCTION

Systemic Lupus Erythematosus (SLE) is a multifactorial chronic systemic autoimmune disease [1]. It is characterized by a lack of immune tolerance to autoantigens such as nuclear antigens [2]. The production of multiple autoantibodies against host DNA and other cellular elements and antigen-antibody complexes would lead to damage to various organs and tissue inflammation [3].

It has been demonstrated that inflammatory mediators and a variety of immune cells, particularly defective T and B cells, that are detrimental components of SLE. Loss of T and B cells tolerance to self-antigens occurs due to the activation of the innate and adaptive immune systems, which is influenced by hormonal, environmental, and genetic variables [4]. The Generation of autoantibodies by autoreactive B cells in response to self-antigens, which sets off an extreme inflammatory response, is the defining feature of systemic lupus erythematosus (SLE) [5]. Tissue damage caused by autoantibodies or immune complex deposits increases morbidity and mortality. Organs and systems affected include the kidneys, heart, arteries, central nervous system, skin, lungs, muscles, and joints [6]. Th cells, or CD4+ T helper cells, are particularly significant for the maturation of the adaptive immune system. Th cells are categorized into many subtypes, including Th1, Th2, Th17 cells, and Treg cells, based on the specific cytokines they generate [7]. Cytokines are low-weight soluble proteins that are produced by different cells in the innate and adaptive immune system. They mediate activation or functional regulation of the immune system by binding to cell surface receptors [8]. The knowledge of the cytokine profiles in SLE not only provides new insight into the pathogenesis of SLE but also sheds light on various clinical applications. Some cytokines, such as interleukin 6 (IL-6), interleukin 10 (IL-10), interferon alpha (INF-a), and Tumor Necrosis Factor-alpha (TNF-a) can serve as biomarkers to monitor disease activity and predict disease severity [9]. The TNF alternatively referred to as TNF-α, is a cytokine composed of fifteen TNF-related proteins. The specific role of TNF α in autoimmunity is not yet fully understood however, partially, in a complex disease such as SLE. Through the engagement of the TNF receptor 1 (TNFR1) and TNF receptor 2 (TNFR2), both the two variants, soluble and transmembrane TNF α, can exert multiple biological effects according to different settings. They can either function as immune regulators, impacting B , T and dendritic cell activity, modulating the autoimmune response, or as pro inflammatory mediators, regulating the induction and maintenance of inflammatory processes in SLE [10].

It is obvious that TNF-α disorder is related to the tissue damage exists in lupus organ failure, death of lymphocyte, and the disability of apoptotic cell clearance. Accordingly, self-antigens were presented and the autoantibodies were developed [10].

https://doi.org/10.32792/utq/utjsci/v11i2.1197

II. METHODOLOGY

A. Collecting Blood Samples

B. Serological Test TNF- α

For an enzyme-linked immunosorbent assay (ELISA), this reagent is intended to be utilised. A human TNF-α antibody has been applied to the plate in advance. TNF-α binds to coated antibodies in sample wells. The sample is then incubated with a biotinylated human TNF-α antibody, which binds to the protein. Next, add streptavidin-HRP, which binds to the antibody biotinylated TNF-α. During the washing phase, unbound streptavidin-HRP is removed following incubation. The concentration of human TNF-α influences the color change after the addition of the substrate solution. By adding acidic stop solution, the process is ended, and the absorbance is measured at 450 nm.

C. Immunological test

Blood samples, approximately 2 milliliters in an EDTA tube, were utilized to measure the number of lymphocytes, basophils, neutrophils, monocytes, eosinophils, and granulocytes using the CBC device (HUMACOUNT 30/Germany).

D. Statistical Analysis

The statistical software package SPSS software kit (version 20) was used to examine the experimental data and estimate the variability in the study's outcomes. Statistical significance was considered as a P-value less than 0.05. The data processing included the application of L.S.D. and other statistical tests.

III. RESULTS

A. Serological Parameter

The results of the present investigation showed that TNF-a levels had increased. It was 106.9± 10.8 greater than those in the control group 52.86 as shown in (Table 1).

TABLE I. Concentration of TNF in the studies groups

Immune Parameters	Cases No.	TNF
		Mean ± S. D
SLE	25	106.9 ± 10.8
Control	25	52.86 ± 3.89
p. value		< 0.001**
LSD		23.6

The different letters indicate significant differences at the level (p<0.05.)

L.S.D least significant difference

B. Immunological Parameter

The results of this study clearly showed that, in comparison to the healthy control group, there was a decrease in the number of lymphocytes, monocytes, basophils, and eosinophils and an increase in the number of immature granulocytes and neutrophils. These results are shown in Tables 2 and 3.

TABLE II. Count of WBC differential in the studies group

	Cases No.	Eosinophile	Basophile	Immature granulocyte
		Mean ± S. D		
SLE	25	0.010 ± 0.003	0.022 ± 0.004	2.68 ± 0.46
Control	25	0.228 ± 0.014	0.029 ± 0.003	0.42 ± 0.47
LSD		0.007	0.083	0.77

TABLE III. Count of WBC differential in the studies groups

	Cases No.	Neutrophile	Lymphocyte	Monocyte
		Mean ± S. D		
SLE	25	7.43 ± 1.66	1.71 ± 0.66	0.21 ± 0.047
Control	25	2.41 ± 0.49	2.50 ± 0.43	0.53 ± 0.025
p. value		<0.001**	< 0.001**	< 0.001**
LSD		0.77	0.22	0.12

The different letters indicate significant differences at the level (p<0.05.)

L.S.D least significant difference

IV. DISCUSSION

A. Serological Parameter

Systemic Lupus Erythematosus (SLE) is a prototypical autoimmune disease characterized by a variety of autoantibodies [11]. The pathologic events in SLE are mediated by the formation of immune complexes [12]. The SLE pathogenesis is characterized by unbalanced pro- and anti-inflammatory cytokine levels. The significance of proinflammatory cytokines in the development of SLE is a subject of debate. The study's findings, which are shown in Table 1, indicate that patients' TNF-α levels significantly increased at the probability level (106.9 ± 10.8) when compared to healthy individuals. This result was in line with research from Mansoura University in Egypt [9], as well as research from Kuwait [13] and India [14]. The TNF-a is generated as a timer on the surface of the cell as a soluble form when the macrophages and dendritic cells are activated [8]. When TNF-a (DC) in the peripheral blood is reduced, levels of soluble TNF receptors are increased . Therefore, this indicated that TNF has a fundamental role in the observed DC modifications [15]. [16] indicated that TNF-a represents an activating cytokine and a maturation factor of dendritic cells that are fundamental for immunological control and related to autoimmunity and SLE. Moreover, [17] presented the case that TNF is a cytokine which plays two key roles in SLE, a proinflammatory mediator and an important immunological regulator.

B. Immunological parameter

The present study shows that neutrophil and immature granulocyte elevated count (7.43 ± 1.66 and 2.68 ± 0.46) respectively. They reduced lymphocyte count1(71 ± 0.66) markedly and this result agreed with [18] and [19]. Furthermore, it is displayed that neutrophils perform an important function in the pathophysiology of SLE through

the neutrophil extracellular traps induction through which the expulsion of nuclear and cytosolic detritus from deceased neutrophils are resulted [20]. It was hypothesized that low-density granulocytes (LDGs), a neutrophil subset abundant promote NETs in those who suffer from SLE. NET formation can also be effected with LDGs [21]. Another finding of the current research is that the basophils and eosinophils levels also are decreased (0.022 ± 0.004 and 0.010 ± 0.003) respectively, which support the findings of other authors' investigations [22] and [18]. It is indicated that the steroid therapy leads to the reduction of monocytes and eosinophils numbers. Under suitable condition, basophil numbers are also decreased in SLE [23]. In addition to their functions in allergy and antiphrastic immunity, basophils infouence influence Th2 polarization, B-cell activation, differentiation, survival, and autoantibody synthesis in numerous autoimmune diseases. According to our results in this study, there is a reduction in monocyte numbers 0.21 ± 0.047 which agree with the study result of [24]. Monocyte phenotypes and functions are changeable in SLE patients so they have limited ability in distinguishing accessory kinds of monocytic cells.

V. Conclusion

This study concludes that SLE patients showed increased TNF-α levels compared to healthy controls, indicating disease activity and associated symptoms such as lupus nephritis and cardiovascular disease. It is identified that lupus neutrophil accumulation is responsible for the disability of the complement pathway in eliminating them. Lastly, it is shown that reduced absolute eosinophil, basophil, and monocyte counts are the outcome of steroid therapy.

Acknowledgment:

We are deeply grateful to all the people who helped us, especially the staff of AL-Hussein Teaching Hospital.

Conflict of interest

Authors declare that they have no conflict of interest.

References

[1] B. Sojod, C. Pidorodeski Nagano, G. M. Garcia Lopez, A. Zalcberg, S. M. Dridi, and F. Anagnostou, "Systemic lupus erythematosus and periodontal disease: a complex clinical and biological interplay," *Journal of Clinical Medicine*, vol. 10, no. 9, p. 1957, 2021.

[2] K. Horst et al., "Antigen presentation, autoantibody production, and therapeutic targets in autoimmune liver disease," *Cellular & Molecular Immunology*, vol. 18, no. 1, pp. 92-111, 2021.

[3] J. Mucke et al., "LUPUS-BEST—treat-to-target in systemic lupus erythematosus: study protocol for a three-armed cluster-randomised trial," *Lupus Science & Medicine*, vol. 8, no. 1, p. e000516, 2021.

[4] B. Dema and N. Charles, "Advances in mechanisms of systemic lupus erythematosus," *Discovery Medicine*, vol. 17, no. 95, pp. 247-255, 2014.

[5] A. Bag-Ozbek and J. S. Hui-Yuen, "Emerging B-cell therapies in systemic lupus erythematosus," *Therapeutics and clinical risk management*, pp. 39-54, 2021.

[6] F. Fanouriakis, N. Tziolos, G. Bertsias, and D. T. Boumpas, "Update on the diagnosis and management of systemic lupus erythematosus," *Annals of the Rheumatic Diseases*, vol. 80, no. 1, pp. 14-25, 2021.

[7] T. Koga et al., "The role of IL-17 in systemic lupus erythematosus and its potential as a therapeutic target," *Expert Review of Clinical Immunology*, vol. 15, no. 6, pp. 629-637, 2019.

[8] D. Y. H. Yap and K. N. Lai, "Cytokines and their roles in the pathogenesis of systemic lupus erythematosus: from basics to recent advances," *BioMed Research International*, vol. 2010.

[9] A. Sabry *et al.*, "Proinflammatory cytokines (TNF-α and IL-6) in Egyptian patients with SLE: its correlation with disease activity," *Cytokine*, vol. 35, no. 3-4, pp. 148-153, 2006.

[10] F. Ghorbaninezhad et al., "Tumor necrosis factor α in systemic lupus erythematosus: Structure, function and therapeutic implications," *International Journal of Molecular Medicine*, vol. 49, no. 4, pp. 1-13, 2022.

[11] D. S. Pisetsky, "Anti-DNA and autoantibodies," *Current Opinion in Rheumatology*, vol. 12, no. 5, pp. 364-368, 2000.

[12] S. Al-Mutairi, A. Al-Awadhi, R. Raghupathy, H. Al-Khawari, P. Sada, A. Al-Herz, and P. Rawoot, "Lupus patients with pulmonary involvement have a pro-inflammatory cytokine profile," *Rheumatology International*, vol. 27, pp. 621-630, 2007.

[13] V. Umare et al., "Effect of proinflammatory cytokines (IL-6, TNF-α, and IL-1β) on clinical manifestations in Indian SLE patients," *Mediators of Inflammation*, vol. 201.

[14] M. A. Gill et al., "Blood dendritic cells and DC-poietins in systemic lupus erythematosus," *Human Immunology*, vol. 63, no. 12, pp. 1172-1180, 2002.

[15] K. Palucka et al., "The interplay of dendritic cell subsets in systemic lupus erythematosus," *Immunology and Cell Biology*, vol. 80, no. 5, pp. 484-488, 2002.

[16] M. Aringer and J. S. Smolen, "SLE-complex cytokine effects in a complex autoimmune disease: tumor necrosis factor in systemic lupus erythematosus," *Arthritis Res Ther*, vol. 5, pp. 1-6, 2003.

[17] Z. Yang et al., "Comparisons of neutrophil-, monocyte-, eosinophil-, and basophil-lymphocyte ratios among various systemic autoimmune rheumatic diseases," *Apmis*, vol. 125, no. 10, pp. 863-871, 2017.

[18] B. K. Han et al., "Neutrophil and lymphocyte counts are associated with different

immunopathological mechanisms in systemic lupus erythematosus," *Lupus Science & Medicine*, vol. 7, no. 1, 2020.

[19] V. Brinkmann et al., "Neutrophil extracellular traps kill bacteria," *Science*, vol. 303, no. 5663, pp. 1532-1535, 2004.

[20] C. Lood et al., "TLR7/8 activation in neutrophils impairs immune complex phagocytosis through shedding of FcgRIIA," *Journal of Experimental Medicine*, vol. 214, no. 7, pp. 2103-2119, 2017.

[21] K. Dossybayeva, D. Abdukhakimova, and D. Poddighe, "Basophils and systemic lupus erythematosus in murine models and human patients," *Biology*, vol. 9, no. 10, 2020.

[22] F. Bashal, "Hematological disorders in patients with systemic lupus erythematosus," *The Open Rheumatology Journal*, vol. 7, p. 87, 2013.

[23] G. Steinbach, P. M. Lynch, R. K. Phillips, M. H. Wallace, E. Hawk, G. B. Gordon, *et. al.*, "The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis", 2000 Jun 29;342(26):1946-52.