AL-KUFA JOURNAL FOR BIOLOGY
ISSN (Print): 2073-8854 **ISSN (online):** 2311-6544. 2024.16 (2), 89-94

Original Research Paper

Evaluate Interleukin-15 in SLE patients and its association with hemolytic anemia

Haider abdul Hussein Zuboon[1*]
Muslim Idan Mohsin[2]

[1,2] *Department of Pathological laboratory analysis, Faculty of Science, University of kufa, Kufa, Iraq.*

Article history
Received: 01/06/2024
Revised: 23/07/2024
Accepted: 25/07/2024

*Corresponding Author:
Email:
Muslim.aljabri@uokufa.edu.iq

Abstract: Systemic Lupus Erythematosus (SLE) is a clearly heterogeneous autoimmunity disorder with an unknown etiology that largely affects several body regions. Interleukin-15 (IL-15) is a cytokine that plays a vital role in the immune regulation and has been related in the pathogenesis of SLE disease. Hemolytic anemia is one of the hematological features of SLE, where the immune system breaks erythrocytes (red blood cells), causing anemia. The aim of this research is to evaluate IL-15 in SLE patients and their correlation with hemolytic anemia to see if this can be used as a biomarker for hemolytic anemia in SLE patients. This study analyzed the demographic and clinical presentation of 200 cases with whole blood and serum from this disease. ELISA and qPCR were also used to measure the amount of IL-15 at the gene and serum of SLE disease association hemolytic anemia. The results showed that IL-15 contributes to the immune response in hemolytic anemia from SLE. The data demonstrated that the gene and serum concentration of IL-15 in SLE disease patients associated with hemolytic anemia were higher than non-hemolytic anemia. In conclusion, the level of serum IL-15 was confirmed to be significantly higher in SLE patients associated with hemolytic anemia, not only in SLE disease patients.

Keywords: SLE disease, IL-15, hemolytic anemia, cytokines disturbances

1.Introduction

Systemic Lupus Erythematosus (SLE) is a persistent autoimmune disease caused via mistakenly attacking the body's tissues in different areas of the body. This can result in inflammation and damage to different organs, including the skin,kidneys, and joints [1]. An autoimmune hemolytic anaemias (AIHAs) is uncommon red blood cells disorder produced by anti-RBCs autoantibodies which cause a short life span of red blood cell [2]. The causes of disease are unclear, but environmental and genetic factors trigger immune responses, leading to overproduction of harmful auto-antibodies and cytokine imbalances, causing tissue and organ damage [3]. SLE always occurs among adolescents and young adults, between the ages of 15 and 44, and primarily affects females in their reproductive years [4]. clinical manifestation of SLE include fever, fatigue,headache, rash, and multi arthritis, also anemia, leukopenia, and thrombocytopenia to vasculitis, serositis, and nephritis extend to serious organ and become life-threatening[5].SLE diagnosis is based on a mix of typical clinical symptoms and signs and positive immunological tests. The diagnosis tends to be difficult or delayed, and it requires good clinical skills for linking clinical and immunological test results [6]. Clinical manifestations of many parts of the body and organs, as well as immunological markers like anti-nuclear antibodies (ANA) and specific antibodies to dsDNA, are the approved way to diagnose SLE [7]. Chloroquine, glucocorticosteroids, and immunosuppressant drugs are classical treatments for SLE patients, while biologic therapy, for example belimumab, is the first confirmed biologic agent for SLE treatment [8]. The imbalances of innate to adaptive immunity leading to a loss of immune tolerance. This loss of tolerance is regulated by a variou of mechanisms, such as cell death (apoptosis), inability to compete for T cell assistance, and failure to differentiate into effector cells [9]. B-lymphocytes contribute to the

DOI: 10.36320/ajb/v16.i2.16840

immunopathogenesis of SLE disease by playing a immportant role in the generation of auto-antibodies [10]. T-cells crucially contribute to SLE immunopathogenesis by stimulating B-cell production of pathogenic auto-antibodies, promoting differentiation, proliferation, and class-switching of produced auto-antibodies [11]. IL-15 is a pro-inflammatory cytokine involve in the development, proliferation, and activation of lines of multiple lymphocytes that use a diversity of signaling pathways [12]. IL-15 is expressed at the mRNA level in musculoskeletal tissue at a high level. This cytokine is produced mainly via monocytes, macrophages, activated CD4+ T-cells, and other cell types like cardiac muscle and kidney as a mature protein[13]. Serum levels of IL-15 were higher in SLE patients and increased in active disease than in inactive (remission) disease [14]. The purpose of this research was to evaluate serum levels of IL-15 in SLE patients receiving treatment and to analyze the correlation between IL-15 and SLE-disease association with hemolytic anemia. To improve that, it was hypothesized that the test of IL-15 is induced up/down regulate based on SLE disease related to hemolytic anemia.

2.Methodology

Samples collection

The samples were collected between 2nd of September to 24th April, 2024, we collected approximately 200 samples, divided into 165 SLE patients diagnosed based on the College of Rheumatology in America classification criteria for SLE [15] and 35 healthy cases of different ages and sexes. They have been divided into two groups: hemolytic and non-hemolytic anemia cases. Each patient had two samples taken, one of serum and one of whole blood, and both were frozen directly after collection; however, Triozol was added to the whole blood samples to protect the mRNA from degrading before freezing.

Laboratory tests

All samples were tested using immunological tests such as anti-nuclear antibody (ANA), anti-dsDNA antibody (Anti-dsDNA) have been performed to test all specimens. Coomb's test and the total serum bilirubin test (TSB) to classify all specimens according to hemolytic or non-hemolytic anemia, Real time RT-PCR

The whole blood samples were used from hemolytic anemia and non-hemolytic anemia cases. All samples were extracted due to the instruction of RNA kit (Solar-bio Company) to isolate the mRNA. The samples were centrifuged at 200 xg for five minutes, and treated with lysis buffer. The pellet was treated with RPE buffer and then treated 2x with WT buffer. The RNA samples were eluted using elution buffer and kept it at -20 to next steps. The second step was performed by using a reverse transcription kit to generate complementary DNA (cDNA). The PCR was ruined with Primer Design Precision's 2x qPCR SYBER Green Master Mix. Plates with sets of primers and probes for the gene of interest are used as a quality assurance measure in the lab. The 799HT Abi Prism equipment was used to conduct 40 cycles of qPCR on the plate. The fluorescence emission from the product can be used to pinpoint the cycle point using qPCR Target. IL-15 levels are proportional to the Ct value. IL-15 primers are used: F: AGGATTTACCGTGGCTTTGAGT, R: CTGCACTGAAACAGCCCAAA, and GAPDH primers are used: F: TGCACCACCAACTGCTTAGC, and R: GGCATGGACTGTGGTCATGAG. These primers were purchased from the South Korean company Macrogen. Relative expression was normalized by comparison to established reference genes. When comparing relative fold expression differences, the Ct method was applied.

The estimation of human IL-15

All of the samples were tested for IL-15. The serum samples were briefly centrifuged to filter out debris before an instantaneous assay. After reconstituting the standard with 1.0 ml of standard sample diluent to make a stock solution of 2000 pg\ml. Allow the standard to remain for 15 minutes with gentle agitation prior to making dilutions. Pipette 875uL of standard \ sample diluent into 250pg\ml tube, and add 125 uL stock solution of 2000 pg/ml into it to get the high standard of 250 pg/ml. Pipette 500ul of standard \ sample diluent into the remaining tubes. It was incubated at 37 °C for 90 minutes with the plate covered. Three times, 1 x wash buffer is aspirated and used to wash each well. Incubation at 37 °C for a 30 minutes, a 100-ul working solution of biotin-conjugated anti-human IL-15 antibody is added to each well. Three times, 1x wash buffer is used to wash each well. After replacing the adhesive strip on the micro titter plate, 100 u1 of HRP-avidin working solution is poured into each well. After washing 3 times with 1 x wash buffer, the mixture was incubated for 30 minutes at 37 °C. Each well is filled with 100 ul of TMB substrate, which is then gently mixed. The micro-plates are then incubated for 20 minutes at 37°C in a dark location.50 ul of stop solution were added to each well in order to stop the color shift. Using a micro plate reader set at 450 nm, the optical density of every well is determined in 30 minutes. It would be possible to determine the IL-15 content in the mystery sample after gathering all of the data.

DOI: 10.36320/ajb/v16.i2.16840

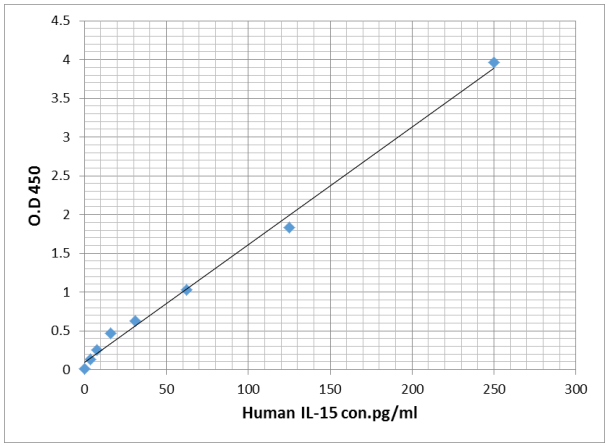

Figure2: The standard curve of interleukin 15 concentration (pg/ml)

Statistical analyses

All graphs and statistical analyses were created and analyzed using GraphPad Prism 9. One- and two-way ANOVA for multiple comparisons were used to detect the specimens with statistically significant differences. The data is shown as a mean standard error of the mean.

3. Results and discussion

The aim of the study to evaluate IL-15 in SLE disease associated with hemolytic anemia cases. First of all, 200 specimens were collected based on variant criteria. Samples have to meet the following criteria between 2nd of September, 2023 to 24th April, 2024. Anti-nuclear antibody and Anti- dsDNA antibody, Coomb's test, and total serum bilirubin test have been performed to test all specimens. The chorus instrument is based on the ELISA technique. The antigen has been embedded in a solid state. Specific immunoglobulins (Igs) bind to antigens during incubation with diluted human serum. The unreacted protein is washed, and then the samples are treated with an anti-human immunoglobulin Abs that has been conjugated to the horseradish peroxidase enzyme. Unbound conjugate antibodies are removed, and peroxidase substrate is added. The color produced is immediately proportional to the titers of the specific antibody present in the serum samples. All the reagents needed to conduct tests on the Chorus/Trio device are involved in the disposables. The gadget used to diagnose SLE disease is expressed in international units (IU/mL) for the results. The result shows that the hemolytic anemia cases is about 10%, and the non-hemolytic anemia cases is about 90%, as shown in Figure 2. It seems that the percentage of non-hemolytic anemia was higher than hemolytic anemia cases. This result was in agreement with[16].

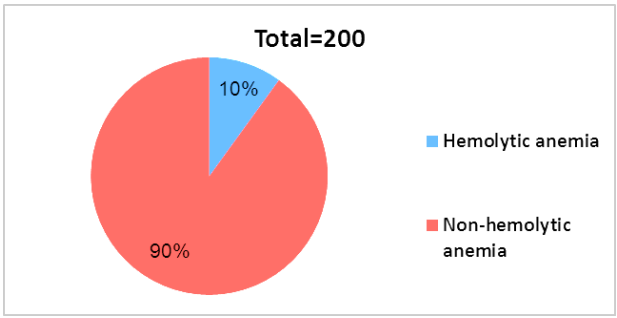

Figure 3: The percentage of hemolytic anemia and non-hemolytic anemia at SLE patients

The results show the percentage of 200 samples from SLE disease that were isolated in different labs Missan province. Coomb's test, and total serum bilirubin tests were used to check all of the samples for hemolytic anemia and non-hemolytic anemia at SLE patients.

The changes of IL-15 at the mRNA level in response to SLE disease association with hemolytic anemia

The aim of this part was to determine whether IL-15 in hemolytic anemia cases was up- and/or down-regulated after SLE disease. In brief, the RNA has been extracted, and cDNA was prepared using a reverse transcriptase kit. After analyzing the data from qPCR, the fold change was calculated using the equation 2^(-ΔΔCt). The results show IL-15 was mostly increased in hemolytic anemia cases at mRNA expression, while IL-15 was not changed in non-hemolytic anemia cases at mRNA expression compared with control cases, as demonstrated in figure 4. The IL-15 was significantly increased with hemolytic anemia group P-value 0.01 **, but there was no significant difference in non-hemolytic anemia cases. These results agreed with those of other researchers, for example, McFarlane and their group found that IL-15 enhances the Janus kinase 3-STAT5 pathway to promote the expansion, proliferation, and cytotoxic function of CD4+ CD28− T cells in SLE patients [17]. It seems that IL-15 can be used as a good marker for detecting the hemolytic anemia cases of SLE disease. These results were similar with another result obtained by [18].

DOI: 10.36320/ajb/v16.i2.16840

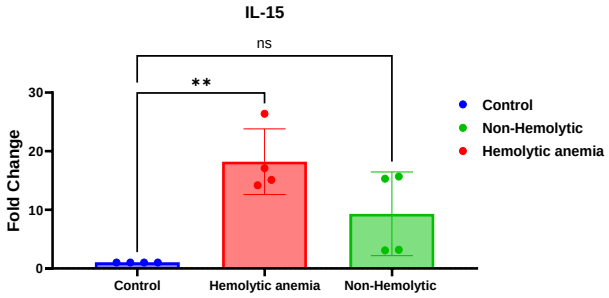

Figure 4: The IL-15 at mRNA expression has changed in response to SLE with hemolytic anemia and non-hemolytic anemia

IL-15 expression has been measured by qPCR in patients who have been detected to have hemolytic anemia in cases of SLE compared with healthy cases. Expression of IL-15 in healthy cases and non-healthy cases was calculated using the 2-ΔΔCt method following estimation of the housekeeping gene GAPDH. IL-15 showed changes in whole blood cells. The significance of differences has been tested by one-way ANOVA, where ** $p<0.01$ is significant and ns = non-significant. The data are the means of 200 samples from three separate experiments with duplicates.

The changes of IL-15 protein in response to SLE disease association with hemolytic anemia cases

The aim of this section was to approve that IL-15 levels are also changed by protein levels and gene expression in hemolytic anemia and non-hemolytic cases of SLE disease. To do this, all samples are collected and classified according to our criteria. The serum was collected and kept at -20 °C from healthy and non-healthy cases of SLE disease. The IL-5 Eliza kit (Solorbio-China) was used according to manufacturer instructions using the sandwich Eliza technique, and the results were analyzed using the standard curve technique. The results showed a significant increase in hemolytic anemia and not a significant difference in the non-hemolytic anemia compared to control cases concentrations. Interestingly, IL-15 significantly increased in the hemolytic anemia group (P-value 0.01 **) compared to control cases, as demonstrated in figure 5. These results agreed with other researchers' for examples; Aringer and his colleagues found that the IL-15 increase with serum level of SLE patient[17] and [18]. It seems that IL-15 can be used as a good marker for detecting the hemolytic anemia cases of SLE disease.

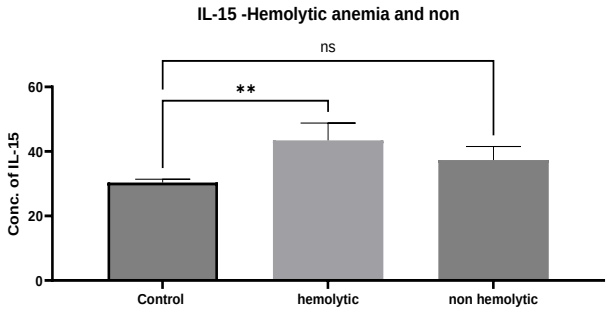

Figure 5: The IL-15 protein expression has changed in response to SLE with hemolytic anemia and non-hemolytic

The IL-15 protein expression has been measured by Eliza in patients who have been detected to have hemolytic anemia in SLE compared with healthy cases. Expression of IL-15 in healthy cases and non-healthy cases was calculated using the standard curve method following an estimation of manufacturer information. IL-15 showed changes in serum. The significance of differences has been tested by one-way ANOVA, where ** $p<0.01$ is significant and ns = non-significant. The data are the means of 200 samples from three separate experiments with duplicates.

Conclusion

IL15 is up-regulated at mRNA levels and protein concentration with SLE associated hemolytic anemia compared with non-hemolytic anemia. It seems that the IL-15 can regulate SLE at different stages of disease especially with hemolytic anemia.

Acknowledgements

The research facilities provided by various hospitals in Missan and Najaf cities have been acknowledged.

Declaration

The writers have no conflict of interest to declare.

Ethics Clearance

This research does not contain any trials with human subjects or animals that were carried out by any of the researchers.

References

1. A. T. Mostafa, A. A. Abd Allah, R.

Hafez, S. Hussien, and R. M. Bakry, "Interleukin-4, and interferon-gamma roles as biomarkers in Systemic Lupus Erythematosus patients.," *Sohag Med. J.*, vol. 26, no. 1, pp. 51–59, 2022.

2. M. Vagace, R. Bajo, and G. Gervasini, "Diagnostic and therapeutic challenges of primary autoimmune haemolytic anaemia in children," *Arch. Dis. Child.*, vol. 99, no. 7, pp. 668–673, 2014.
3. M. A. Ameer, H. Chaudhry, J. Mushtaq, O. S. Khan, and M. Babar, "An Overview of Systemic Lupus Erythematosus (SLE) Pathogenesis , Classification , and Management," vol. 14, no. 10, 2022, doi: 10.7759/cureus.30330.
4. Z. A. Al-Sarray, I. A. Al-Rayahi, A. H. Al-Hafidh, and A. T. Dwayyikh, "Serum Protein Electrophoresis in Iraqi Systemic lupus Erythematous Patient," *Al-Nisour J. Med. Sci.*, vol. 2, no. 1, 2020.
5. J. De Azevedo Silva, C. Addobbati, P. Sandrin-Garcia, and S. Crovella, "Systemic Lupus Erythematosus: Old and New Susceptibility Genes versus Clinical Manifestations," *Curr. Genomics*, vol. 15, no. 1, pp. 52–65, 2014, doi: 10.2174/1389202915011403061137 15.
6. A. Fava and M. Petri, "Systemic lupus erythematosus: Diagnosis and clinical management," *J. Autoimmun.*, vol. 96, no. November, pp. 1–13, 2019, doi: 10.1016/j.jaut.2018.11.001.
7. A. Kuhn, G. Bonsmann, H.-J. Anders, P. Herzer, K. Tenbrock, and M. Schneider, "The diagnosis and treatment of systemic lupus erythematosus," *Dtsch. Arztebl. Int.*, vol. 112, no. 25, p. 423, 2015.
8. F. Basta, F. Fasola, K. Triantafyllias, and A. Schwarting, "Systemic Lupus Erythematosus (SLE) Therapy: The Old and the New," *Rheumatol. Ther.*, vol. 7, no. 3, pp. 433–446, 2020, doi: 10.1007/s40744-020-00212-9.
9. M. Woods, Y. R. Zou, and A. Davidson, "Defects in germinal center selection in SLE," *Front. Immunol.*, vol. 6, no. AUG, pp. 1–7, 2015, doi: 10.3389/fimmu.2015.00425.
10. S. Iwata and Y. Tanaka, "B-cell subsets, signaling and their roles in secretion of autoantibodies," *Lupus*, vol. 25, no. 8, pp. 850–856, 2016, doi: 10.1177/0961203316643172.
11. M. R. W. Barber *et al.*, "Global epidemiology of systemic lupus erythematosus," *Nat. Rev. Rheumatol.*, vol. 17, no. 9, pp. 515–532, 2021.
12. E. Pieterse and J. van der Vlag, "Breaking immunological tolerance in systemic lupus erythematosus," *Front. Immunol.*, vol. 5, no. APR, pp. 1–8, 2014, doi: 10.3389/fimmu.2014.00164.
13. L. S. Quinn and B. G. Anderson, "Interleukin-15, IL-15 receptor-alpha, and obesity: concordance of laboratory animal and human genetic studies," *J. Obes.*, vol. 2011, 2011.
14. S. Lin, M. Kuo, H. Hsiao, P. Lee, and J. Chen, "Activating and inhibitory receptors on natural killer cells in patients with systemic lupus erythematosis-regulation with interleukin-15," pp. 1–20, 2017, doi: 10.1371/journal.pone.0186223.
15. A. E. RHEUMATISM, "Updating the American College of Rheumatology," vol. 40, no. 9, p. 1997, 1997.
16. E. F. Borba, D. B. Araujo, E. Bonfá, and S. K. Shinjo, "Clinical and immunological features of 888 Brazilian systemic lupus patients from a monocentric cohort: Comparison with other populations," *Lupus*, vol. 22, no. 7, pp. 744–749, 2013, doi: 10.1177/0961203313490432.

17. A. McFarlane, E. Pohler, and I. Moraga, "Molecular and cellular factors determining the functional pleiotropy of cytokines," *FEBS J.*, vol. 290, no. 10, pp. 2525–2552, 2023, doi: 10.1111/febs.16420.
18. M. Aringer *et al.*, "Serum interleukin-15 is elevated in systemic lupus erythematosus," *Rheumatology*, vol. 40, no. 8, pp. 876–881, 2001, doi: 10.1093/rheumatology/40.8.876.