ORIGINAL ARTICLE

Interferon alpha and non-specific markers of inflammation in patients with systemic lupus erythematosus

Lamija Zečević[1], Edin Begić[2,3], Buena Aziri[3], Izeta Aganović-Mušinovic[4]

[1]Department of Clinic Biochemistry and Immunology, Clinical Centre, University of Sarajevo, [2]General Hospital "Prim. dr. Abdulah Nakaš", [3]Sarajevo Medical School, Sarajevo School of Science and Technology, [4]Department for Immunology, School of Medicine, University of Sarajevo; Sarajevo, Bosnia and Herzegovina

ABSTRACT

Aim To determine the value of IFN (intzerferon)-α in the patients with systemic lupus erythematosus (SLE) and to correlate IFN-α with values of non-specific biochemical parameters of inflammation (C-reactive protein, leukocytes values, erythrocyte sedimentation rate, albumins and globulins).

Methods Research included 55 patients with SLE diagnosis and a control group consisted of 25 healthy subjects (during period 2019-2020). IFN (Interferon)-α and non-specific biochemical parameters of inflammation were obtained using standard protocols.

Results IFN-α values were independent of gender (p=0.95). The difference in serum IFN-α values in relation with the age in the SLE group was statistically significant (p=0.036). Only serum globulin was significantly higher (p=0.0023) in IFN-α positive compared to IFN-α negative SLE patients. A statistically significant correlation between the values of IFN-α and globulin was proved (r=0.315; p=0.019). No significant correlation was found between other non-specific biochemical parameters and IFN-α values.

Conclusion Increased IFN-α values were observed in younger patients, and the correlation between IFN and globulin was proved.

Key words: immunity, inflammation, interferon-alpha

Corresponding author:
Lamija Zečević
Department of Clinic Biochemistry and Immunology, Clinical Centre
University of Sarajevo
Bolnička 25, 71000 Sarajevo,
Bosnia and Herzegovina
Phone: +387 298-081;
Fax: +387 297-844;
E-mail: zecevicl@hotmail.com
ORCID ID: https://orcid.org/0000-0003-1110-0675

Original submission:
24 January 2022;
Revised submission:
14 February 2022;
Accepted:
10 March 2022
doi: 10.17392/1470-22

Med Glas (Zenica) 2022; 19(2): 207-211

INTRODUCTION

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease with a heterogeneous spectrum of clinical manifestations (1). Recently, it has been suggested that SLE should be defined as a syndrome, due to numerous symptoms resulting from involvement of almost all organs and organ systems (musculoskeletal, skin, kidneys, heart, lungs, central nervous system, gastrointestinal and haematological system) (2). The role of cytokines, as mediators of immune responses, is their mediation in the differentiation, maturation and activation of different cell types (3). Interferon (IFN) is produced on a regular basis in people with SLE, thus sustaining an autoimmune process (4). Interferon type I (IFN-I) consists of IFN-α (13 subtypes), IFN-β, IFN-ε, IFN-κ and IFN-Ω (5). Patients with SLE have increased expression of IFN-type I regulated genes; the risk of disease has been linked to gain-of-function genetic variants in the type I IFN pathway (2,6). Type I IFN, particularly IFN-α, is implicated in preclinical and suspected SLE (7).

IFN-α is a pleiotropic cytokine that can affect multiple cell types involved in SLE (2). Inflammatory activity in SLE patients, as in all other diseases, is measured by non-specific parameters of inflammation: C-reactive protein (CRP), leukocytes values, erythrocyte sedimentation rate, albumins and globulins (2,8). Although they are an essential part of clinical practice, due to their specificity, these parameters are not sufficient for further clinical monitoring of the disease. The low specificity for these parameters is manifested in the inability to distinguish infection from the active state of the disease (2).

Interferon levels help identify people who are at risk and could benefit from early preventive measures (7). In SLE patients, infection is the leading cause of death, accounting for a quarter of all deaths (9). Due to the immune consequences of active disease or treatments with steroids and other immunosuppressants, infectious conditions in SLE patients can be extremely severe (9). Early diagnosis and intervention are critical in preventing infections and improving SLE outcomes (10).

The evaluation of inflammatory disease activity is complicated by the fact that infections occur at the same time as the onset of the disease or during exacerbation (11). The most common are respiratory and urinary tract infections, tuberculosis or opportunistic infections (9). CRP values can be a part of disease activity monitoring in most infectious and autoimmune processes (9). The exception is SLE, in which CRP is normal or discretely elevated in the acute phase of the disease (11,12). The high-sensitivity CRP (hsCRP) allows the measurement of much smaller amounts of this protein and is detected in 77% of SLE patients with active disease (11,12). Values of hsCRP are significantly correlated with disease activity, especially in patients with musculoskeletal and haematological involvements and in patients with serositis (15-17). Moreover, elevated hsCRP levels are generally significantly associated with cardiovascular risk factors (13).

In Bosnia and Herzegovina there are no studies relating to identification markers that could be used for the diagnosis at an early stage of SLE.

The aim of this study was to determine the value of IFN-α in the patients with SLE group and to correlate IFN-α with values of non-specific biochemical parameters of inflammation (C-reactive protein, leukocytes values, erythrocyte sedimentation rate, albumins and globulins).

PATIENTS AND METHODS

Patients and study design

Research included a research group of 55 patients with SLE diagnosis attended to the Dermatology Clinic of Clinical Centre, University of Sarajevo, Bosnia and Herzegovina, during the period 2019-2020, and a control group of 25 randomly selected healthy subjects who corresponded to the SLE group by gender and age and who, based on subjective and objective indicators, did not have clinical symptoms of SLE.

Inclusion criteria were age of or above 18, SLE diagnosis based on 4 of the 11 European League Against Rheumatism (EULAR)/American College of Rheumatology (ACR) SLE classification criteria (2). Exclusion criteria were age under 18, patients with verified human immunodeficiency virus infection or other immune deficiencies, patients with immunotherapy, patients with cancer or metastatic cancer, and patients with acute infectious disease. Inclusion criteria for the control group were age of or above 18, no comorbidities in medical history, and they are randomly selected in patients that came for regular health check-up.

Medical records were analysed and included personal data of patients (age, gender). Patients between the age of 19 and 67 were included in the study. The SLE group was further divided on the basis of the mean age (46 years) into the patient aged 19-46 and 47-67 years.

Values of non-specific biochemical parameters of inflammation (CRP, leukocytes, erythrocyte sedimentation rate, albumins and globulins) and values of IFN-α are part of the common laboratory monitoring of patients with SLE at the Clinic for Immunology, Clinical Centre of the University of Sarajevo, Bosnia and Herzegovina.

An ethical approval was obtained from Ethics Committee of the Clinical Centre of the University of Sarajevo.

Methods

A standardized VeriKine Human Interferon Alpha Multi-Subtype Serum enzyme-linked immunosorbent assays (ELISA) kit (PBL Assay Science, New York, USA) was used to determine serum interferon alpha (IFN-α) values using a microtiter plate with 96-well clear flat bottom, which adsorbed monoclonal antibodies to IFN-α. In each clear flat bottom, standards and patient samples (50 μL) were pipetted according to a previously established order and incubated for one hour. Unbound IFN-α was removed by automatic rinsing on an autowasher BioTek ELx50 (The Lab World Group, Massachusetts, United States). The next step was incubation with Ab-solution (another type of antibody specific for the same antigen), added to each clear flat bottom (100 μL). Elution was followed by conjugate incubation by adding 100 μL of conjugate with horseradish peroxidase. The next phase, the incubation phase of the substrate, was preceded by copious automatic washing, after which 100 μL of tetra-methylbenzidine solution was added for 15 minutes. A colour (blue) was developed in the presence of a chromogenic substrate, with intensity proportional to the concentration of IFN-alpha in the sample. The addition of stop solution (H_2SO_4) stopped the development of colour, and the intensity of the resulting colour (yellow) was read on an automatic microtiter plate reader BioTek ELx800 (The Lab World Group, Massachusetts, United States) at 450 nm wavelength and the results analysed in BioTek Gen5 software (Microsoft Corporation, New Mexico, United States). Due to the specificity of monoclonal antibodies, high sensitivity of the test is ensured, and it is possible to determine very low cytokine concentrations (pg/mL). The assay range was 12.5-1.000 pg/mL.

Statistical analysis

For descriptive statistics measures of central tendency (arithmetic mean and median) and variability (standard deviation and standard error) were used. Student's t-test or Mann-Whitney U test was used to test the difference between the two data groups, depending on the data distribution (first checked by Kolmogorov-Smirnov test or Shapiro-Wilk test). To estimate the correlation, the Spearman correlation coefficient (r) was used. The □2- test of the independence of variables and Fisher test of exact probability for evaluation of an association of categorical variables were applied. The level of $p<0.05$ was accepted as statistically significant.

RESULTS

Females were predominated in both SLE and control group, 51 (92.7%) and 23 (92.0%), respectively.

The differences in the mean values of serum IFN-α concentration between the SLE and control group were not significantly different (p=0.561). In both the SLE and control group, the highest percentage of patients/subjects had negative IFN-α values: in the SLE group four (7.3%) patients had positive IFN-α values, while in the control group, only one (4%) (p=0.95).

The difference in serum IFN-α values between two age groups of SLE patients were statistically significant (p=0.036). Gender was found not to be statistically significantly related to IFN-α values (p=0.267).

Non-specific biochemical parameters of inflammation (C-reactive protein, leukocytes values, erythrocyte sedimentation rate, albumins) did not differ significantly between IFN-α positive patients compared to the IFN-α negative patients. Only serum globulin was significantly higher (p=0.0023) in IFN-α positive SLE patients (23.9±7.67) compared with IFN-α negative SLE patients (13.16±6.25; p=0.0023) (Table 1).

Table 1. Non-specific biochemical parameters of inflammation in relation to IFN-α values in patients with systemic lupus erythematosus (SLE)

Parameter	IFN-α (mean±standard deviation)		p
	Negative (n=51)	Positive (n=4)	
C-reactive protein	4.84±5.24	4.55±2.37	0.912
Leukocytes (x109⁄L)	7.54±2.16	9.98±5.31	0.060
Erythrocyte sedimentation rate (mm⁄h)	24.2o±10.15	23.00±6.98	0.819
Albumins (g⁄L)	37.41±9.42	41.1±11.45	0.460
Globulins (g⁄L)	13.16±6.25	23.9±7.67	0.0023

A moderately strong, statistically significant, positive correlation between values of IFN-α and globulin was proved (r=0.315; p=0.019). No significant correlation was found between other non-specific biochemical parameters and IFN-α values.

DISCUSSION

In this study, the highest percentage of the SLE patients as well as well as the controls had negative serum IFN-α values. Prevalence of positive values of the IFN-α in the SLE group was 7.3% and in the control group 4.0%, resulting in non-significant difference.

An increase of IFN-α activity has also been reported in younger respondents similarly to our study: in a study by Niewold et al. an increase of serum IFN-α values was demonstrated in younger SLE patients (15); but interestingly, authors found no statistically significant gender association with INF-α values (15).

Most SLE patients have spontaneous expression of type I IFN-induced genes in circulating mononuclear cells and peripheral tissues (16). IFN-α expression is amplified in the absence of adequate stimuli, and IFN- α concentration is linked to a disease activity (19). The IFN-α thus has the potential to dramatically affect the development, progression, and pathogenesis of SLE, as it can affect the function and activation of most major immune subpopulations as a link between innate and acquired immunity (16, 20-22).

A statistically significant association between IFN-α and globulin was found in our study; it could be noticed that the average values of certain parameters of inflammation (albumins and globulins) were higher in patients with positive IFN-α compared to those with negative IFN-α. Patients with SLE will have increased antibody production that may appear as hypergammaglobulinemia (24); however, immunoglobulin deficiency is something that will follow the SLE patients (primary, secondary) (25). Almaghlouth et al. concluded that measurement of immunoglobulin levels in SLE patients might be useful to detect patients at risk of bacterial infection (25).

No association between IFN-α and other non-specific biochemical parameters has been demonstrated in our study. IFN-α, have emerged as key pathogenic cytokines in SLE and they are capable of inducing a number of biological effects that can alter the function of effector cells, such as B cells, T cells, or dendritic cells, and represents a therapeutic target.

In conclusion, increased IFN-α values in relation to younger age were found, but gender did not have impact on IFN-α values; the association between IFN-α values and globulin indicated the occurrence of antibody production.

FUNDING

No specific funding was received for this study.

TRANSPARENCY DECLARATION

Competing interests: None to declare.

REFERENCES

1. Connelly K, Morand EF. Systemic lupus erythematosus: a clinical update. Intern Med J 2021; 51:1219-28.
2. Sarwar S, Mohamed AS, Rogers S, Sarmast ST, Kataria S, Mohamed KH, Khalid MZ, Saeeduddin MO, Shiza ST, Ahmad S, Awais A, Singh R. Neuropsychiatric systemic lupus erythematosus: a 2021 update on diagnosis, management, and current challenges. Cureus 2021; 13:e17969.
3. Idborg H, Oke V. Cytokines as biomarkers in systemic lupus erythematosus: value for diagnosis and drug therapy. Int J Mol Sci 2021; 22:11327.
4. Chasset F, Arnaud L. Targeting interferons and their pathways in systemic lupus erythematosus. Autoimmun Rev 2018; 17:44-52.
5. Wittling MC, Cahalan SR, Levenson EA, Rabin RL. Shared and unique features of human interferon-beta and interferon-alpha subtypes. Front Immunol 2021; 11:605673.
6. Rönnblom L, Leonard D. Interferon pathway in SLE: one key to unlocking the mystery of the disease. Lupus Sci Med 2019; 6:e000270.
7. Rubio J, Kyttaris VC. Measuring IFN activity in suspected SLE: a valuable step? Expert Rev Clin Immunol 2021; 17:545-8.

8. Uzkeser H, Keskin H, Haliloglu S, Cayir Y, Karaaslan Y, Kosar A, Carlioglu A. Is mean platelet volume related to disease activity in systemic lupus erythematosus? Int J Clin Pract 2021; 75:e14676.
9. Barber MRW, Drenkard C, Falasinnu T, Hoi A, Mak A, Kow NY, Svenungsson E, Peterson J, Clarke AE, Ramsey-Goldman R. Global epidemiology of systemic lupus erythematosus. Nat Rev Rheumatol 2021; 17:515-32.
10. Justiz Vaillant AA, Goyal A, Bansal P, Varacallo M. Systemic Lupus Erythematosus. Treasure Island (FL): StatPearls Publishing, 2021.
11. Enocsson H, Gullstrand B, Eloranta ML, Wetterö J, Leonard D, Rönnblom L, Bengtsson AA, Sjöwall C. C-reactive protein levels in systemic lupus erythematosus are modulated by the interferon gene signature and CRP gene polymorphism rs1205. Front Immunol 2021; 11:622326.
12. He S, Tang C, Yu J, Ma J, Qiao M, Zhou W, Chen Y, Zhang X. Combining C reactive protein and serum albumin to predict 90-day mortality in systemic lupus erythematosus with serious community-acquired infections. Lupus Sci Med 2021; 8:e000505.
13. Hernández-Martínez A, Gavilán-Carrera B, Vargas-Hitos JA, Morillas-de-Laguno P, Sola-Rodríguez S, Rosales-Castillo A, Artero EG, Sabio JM, Soriano-Maldonado A. Ideal cardiovascular health in women with systemic lupus erythematosus: association with arterial stiffness, inflammation, and fitness. Int J Cardiol 2021; 330:207-13.
14. Mathian A, Koutouzov S. Interferon-alpha: a key cytokine in systemic lupus erythematosus pathogenesis. Rev Med Interne 2008; 29:696-700.
15. Niewold TB, Clark DN, Salloum R, Poole BD. Interferon alpha in systemic lupus erythematosus. J Biomed Biotechnol 2010; 2010:948364.
16. Cucak H, Yrlid U, Reizis B, Kalinke U, Johansson-Lindbom B. Type I interferon signaling in dendritic cells stimulates the development of lymph-node-resident T follicular helper cells. Immunity 2009; 31:491–501.
17. Reizis B, Colonna M, Trinchieri G, Barrat F, Gilliet M. Plasmacytoid dendritic cells: one-trick ponies or workhorses of the immune system? Nat Rev Immunol 2011; 11:558– 65.
18. Kirou KA, Gkrouzman E. Anti-interferon alpha treatment in SLE. Clin Immunol 2013; 148:303-12.
19. Abida R, Yeoh SA, Isenberg DA. Advances in systemic lupus erythematosus. Medicine 2022; 50:7-17.
20. Ito T, Kanzler H, Duramad O, Cao W, Liu YJ. Specialization, kinetics, and repertoire of type 1 interferon responses by human plasmacytoid predendritic cells. Blood 2006; 107:2423-31.
21. Lee JY, Park JK, Lee EY, Lee EB, Song YW. Circulating exosomes from patients with systemic lupus erythematosus induce a proinflammatory immune response. Arthritis Res Ther 2016; 18:264.
22. Riboldi P, Gerosa M, Moroni G, Radice A, Allegri F, Sinico A, Tincani A, Meroni PL. Anti-DNA antibodies: a diagnostic and prognostic tool for systemic lupus erythematosus? Autoimmunity 2005; 38:39-45.
23. Eudy AM, Vines AI, Dooley MA, Cooper GS, Parks CG. Elevated C-reactive protein and self-reported disease activity in systemic lupus erythematosus. Lupus 2014; 23:1460-7.
24. Draborg AH, Lydolph MC, Westergaard M, Olesen Larsen S, Nielsen CT, Duus K, Jacobsen S, Houen G. Elevated concentrations of serum immunoglobulin free light chains in systemic lupus erythematosus patients in relation to disease activity, inflammatory status, B cell activity and Epstein-Barr virus antibodies. PLoS One 2015; 10:e0138753.
25. Almaghlouth I, Johnson SR, Pullenayegum E, Gladman D, Urowitz M. Immunoglobulin levels in systemic lupus erythematosus: a narrative review. Lupus 2021; 30:867-75.