Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.47750/jptcp.2023.30.05.025

PD-1 and NFATc1 as promising immunotherapy for SLE patients

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Submitted: 17 January 2023; Accepted: 10 February 2023; Published: 05 March 2023

ABSTRACT

Background: Systemic lupus erythematosus (SLE) is a chronic, autoimmune disease, with a wide range of clinical symptoms. This study aims to investigate PD-1 and NFATc1 gene expression in SLE patients.

Methodology: In this study, 50 diagnosed SLE patients (25 females as early diagnosed SLE without treatment, 25 females as SLE patients under treatment with (prednisolone, hydroxychloroquine) and 25 healthy individuals as control whose ages arranges from 20 to 45 years were included. The gene expression level of PD-1 and NFATc1 were assessed by real-time polymerase chain reaction (RT-PCR).

Results: A PD-1 gene expression level (folds) results showed a significant increase in the treated group (59.74 ± 33.26 folds) compared to early diagnosed group (23.18 ± 10.17 folds) and control group (3.46 ± 1.93 folds), and early diagnosed group showed a non-significant increase compared to control group. The NFATc1 gene expression level (folds) results showed a significant increase in the treated group (0.17 ± 0.16 folds) compared to early diagnosed group (0.003 ± 0.002 folds) and control group (0.001 ± 0.0007 folds), and early diagnosed group showed a non-significant increase compared to control group.

Conclusions: Based on these results, PD-1 and NFATc1 is important in the SLE pathogenesis. These results may hold promise for developing a new SLE immunotherapy by focusing on PD-1 and NFATc1.

Keywords: *Systemic lupus erythematosus, NFATc1, PD-1*

INTRODUCTION

SLE is a severe autoimmune disease. Clinical symptoms of this disease can be mild, transient, or fatal [1]. It is characterized by persistent inflammation and the generation of autoantibodies, which can impact various organs in the body [2, 3]. This disease is multifactorial and is known for an imbalance of the innate and adaptive immune systems brought on by genetic susceptibility and environmental variables [4]. Before the onset of clinical symptoms, the development of autoantibodies against self-antigens may be the disease etiology [5]. Autoantibodies play a pivotal role in the immunological dysfunction observed in SLE [6]. Lack of immunological tolerance to autoantigens like nuclear antigens is a characteristic of SLE [7,8].

SLE patients often suffer from impaired antiviral immunity and are highly vulnerable to develope life-threatening opportunistic infections, which are the main cause of death [9]. SLE is estimated to affect five million people globally, 90% are reproductive age women [10]. The surface protein known as CD279, or "programmed cell death protein 1,"is a member of the immunoglobulin superfamily and functions as an immunomodulatory molecule". Inhibitory signals are delivered by PD-1 when it binds with its ligand PD-L1, which negatively regulates the immunological response following T cell activation and maintains the immune tolerance equilibrium [11]. To stop autoimmune tissue damage, the immune checkpoint molecule PD-1 down-regulates T-cell activation during immunological responses. Long-term antigen exposure leads to PD-1 expression in tumors and chronic infections, which may impair the immune system's ability to eliminate pathogens or deteriorated cells [12]. The use of checkpoint inhibitors (PD-1, PD-L1) is revolutionizing the process to managing oncological diseases [13]. In a number of experimental animal models, such as for SLE, type 1 diabetes (T1D), and multiple sclerosis (MS), rheumatoid arthritis (RA), PD-1 has been demonstrated to maintain the immune system's balance and prevent autoimmunity in different ways [14]. The PD-1 axis in SLE regulates the innate and adaptive immune system subsets [15]. Immune-regulatory molecules such as the PD-1 receptor and its ligands (PD-L1, PD-L2) are connected to the emergence of SLE [16]. The therapy of some tumors involves the use of antibodies that target PD-1 receptors and prevent their activation [17]. The clinical signs of autoimmune disease, such as SLE, may also be brought on by this antibody therapy [18,17]. Polymorphisms in the PD-1 gene have appeared in SLE. PD1.3 and PD1.5 polymorphisms are related to SLE and lupus nephritis vulnerability [16,19]. Only a few research, meanwhile, have up to this point hinted to a potential connection between SLE and PD-1 gene expression. On the other hand, there is another protein which relationship has not been studied for SLE in the literature, yet it is significant in immune response, which is NFATc1. NFATc1 is a transcription nuclear factor of activated T cells (NFAT) family [20]. NFATc1 has been connected to T-cell inducing cell cycle arrest and death [21]. In addition to having lower levels of IgG1 and IgE, mice lacking NFATc1 also produce less IL-4 and Th2 cytokines [22]. According to several studies, NFATc1 plays a role in (Th17) response and it is crucial for T-cell development. Due to the unique functions that different family members have in proliferating and activating T-cell, NFAT inhibitors have been utilized in organ transplantation to reduce transplant rejection and to manage autoimmune diseases [23]. The study aims to investigate PD-1 and NFATc1 gene expression in SLE patients.

METHODOLOGY

Subject collection

From March 2022 to August 2022, this work was performed in the department of rheumatology at Al-Yarmouk Teaching Hospital and the Medical City (Baghdad Teaching Hospital) in Baghdad. included 75 females aged between 20 to 45 years old, it includes 25 females as early diagnosed SLE (without treatment) and 25 females as SLE patients under treatment with (prednisolone, hydroxychloroquine), and 25 females healthy individuals as control. All patients were diagnosed according to clinical examination by a rheumatologist physician and selected based on the SLE revised classification criteria published by the American College of Rheumatology (ACR) [24]. Patients with other diseases (Hypertension, cardiovascular disease, diabetes types I, II) or any other chronic disease including autoimmune diseases were excluded from this study.

Blood collection

All participant patients and control had 0.25 ml of their intravenous blood (whole blood) drawn, and this was added to a 0.75 ml TRIzolTM Reagent in eppendorf tube. By repeatedly mixing up and down, the lysate was homogenized.

Real time PCR Primers

The primers were provided by (Macrogen. USA). The β-Globin primer (Housekeeping) was designed according to Mosaferi et al. [25] and

PD-1 primer was designed according to Ghorbani et al, [26], while NFATc1 primer is specially designed for this study by using (Primer 3) program, as shown in the table(1).

TABLE 1: Design of PD-1, NFATc1 and β-Globin primers.

Name of gene	Primer	Sequences (5′→3′ direction)	Primer size	Reference
PD-1	Forward	TAGAGAAGTTTCAGGGAAGG	20	Ghorbani et al, [25]
	Reverse	ATGTGTAAAGGTGGAGGG	18	
NFATc1	Forward	CACCAAAGTCCTGGAGATCCCA	22	(Primer3) program
	Reverse	TTCTTCCTCCCGATGTCCGTCT	22	
β-Globin	Forward	ACACAACTGTGTTCACTAGC	20	Mosaferi et al. [26]
	Reverse	CAACTTCATCCACGTTCACC	20	

Extraction of RNA

TRIzolTM Reagent's protocol (Thermo Scientific, USA) were followed to isolate RNA from the samples.

RT-PCR and gene expression

The One Step RT- qPCR kit, (Promega, USA made) used, contains qPCR Master Mix 5 µl, RT mix 0.25 µl MgCl2 0.25 µl, Forward primer 0.5 µl, Reverse primer 0.5 µl, Nuclease Free Water 1 µl, Final Total Volume 10 µl. Add 1 µl of Template and 9 µl of Master mix to an aliquotor for a single reaction.

Next, it was put into a Real-time PCR. The first step involved only a cycle lasting 15 min at 37 °C for c DNA and the preliminary denaturation taking place for 5 min at a temperature of 95°C. Step 2 involved 40 cycles, which included the following: A: 20 s at 95 °C to denaturation of template, B: 20 s at 60°C, 55 °C, and 65°C for PD-1, NFATc1 and β-globin respectively, for the primers for binding to template annealing, for the related primers to be lengthened, it takes 20 s at 72°C. Step 3: Melted green (three cycles for one s at 72 °C to 95 °C). By using the Livak Method, determine the gene expression levels as indicated in:-

Folding =2-ΔΔCT

ΔCT =CT gene - CT House Keeping gene

ΔΔCT =ΔCT Treated or Control - ΔCT Control

Statistical Analysis

SPSS Statistical software (IBM SPSS 26.0) was used to examine the data. employed the least significant difference (LSD) test with a lower probability than 0.05 ($p < 0.05$).

RESULTS

A PD-1 gene expression level (folds) results showed a significant increase in the treated group (59.74 ± 33.26 folds) compared to early diagnosed group (23.18 ± 10.17 folds) and control group (3.46 ± 1.93 folds). Early diagnosed group showed a non-significant increase compared to control group, as shown in the tables (2) and (3).

TABLE 2: Fold of PD-1 expression depending on 2-ΔΔCt method

Study groups	Mean of PD-1 Ct	Mean of PD-1 ΔCt	Mean of ΔCt of β-globulin	Mean of PD-1 ΔΔCt	2-ΔΔCt	Mean of PD-1 gene folding
Control	21.17	9.31	11.86	-0.0007	3.46	3.46 ± 1.93 B
Treated patients	20.66	6.40	14.27	-2.92	59.74	59.74 ± 33.26 A
Early diagnosed patients	20.33	7.35	12.98	-1.96	23.18	23.18 ± 10.17 B
P-value (P<0.05)	-	-	-	-	-	0.146
LSD	-	-	-	-	-	37.53
* Different letters denote to the significant difference at P<0.05.						

TABLE 3: Fold of PD-1 gene in Ct, ΔCt and 2-ΔCt value

Study groups	Mean ± SE of PD-1 Ct	Mean ± SE of Ct of β-globulin	Mean ± SE of PD-1 ΔCt	Mean ± SE of PD-1 2-ΔCt
Control	21.17 ± 0.91	11.86 ± 0.76	9.31 ± 0.60	0.005 ± 0.003
Treated patients	20.66 ± 0.86	14.27 ± 0.86	6.40 ± 0.93	0.09 ± 0.05
Early diagnosed patients	20.33 ± 0.65	12.98 ± 0.72	7.35 ± 0.78	0.04 ± 0.02
P-value (P<0.05)	0.765	0.026	0.036	0.144
LSD	0.56	2.38	1.94	0.06

NFATc1 gene expression level (folds) results showed a significant increase in the treated group (0.17 ± 0.16 folds) compared to early diagnosed group (0.003 ± 0.002 folds) and control group (0.001 ± 0.0007 folds). Early diagnosed group showed a non-significant increase compared to control group, as shown in the tables (4) and (5).

TABLE 4: Fold of NFATc1 expression depending on 2-ΔΔCt method

Study groups	Mean of NFATc1 Ct	Mean of NFATc1 ΔCt	Mean of ΔCt of β-globulin	Mean of NFATc1 ΔΔCt	2-ΔΔCt	Mean of NFATc1 gene folding
Control	23.98	12.12	11.86	10.84	0.001	0.001 ± 0.0007B
Treated patients	24.68	9.24	14.27	7.97	0.17	0.17 ± 0.16A
Early diagnosed patients	25.12	12.14	12.98	10.86	0.003	0.003 ± 0.002B
P-value (P<0.05)	-	-	-	-	-	0.338
LSD	-	-	-	-	-	0.11
* Different letters denote to the significant difference at P<0.05.						

TABLE 5: Fold of NFATc1 gene in Ct, ΔCt and 2-ΔCt value

Study groups	Mean ± SE of NFATc1 Ct	Mean ± SE of Ct of β-globulin	Mean ± SE of NFATc1 ΔCt	Mean ± SE of NFATc1 2-ΔCt
Control	23.98 ± 0.46	11.86 ± 0.76	12.12 ± 0.44	0.0 ± 0.0
Treated patients	24.68 ± 0.47	14.27 ± 0.86	9.24 ± 0.90	0.070 ± 0.066
Early diagnosed patients	25.12 ± 0.69	12.98 ± 0.72	12.14 ± 0.70	0.001 ± 0.0009
P-value (P<0.05)	0.343	0.026	0.007	0.339
LSD	0.76	2.38	1.91	0.114

DISCUSSION

PD-1 gene expression

When PD-1,interacts with its ligands, the negative T cell regulator PD-1 causes negative signals to be sent to T cells, making it a possible gene in the development of SLE [27]. PDCD1 gene encodes the PD-1 molecule and functions to maintain peripheral tolerance by adverse regulation of the self-reactive T- and B-cells. There is a chance that the PDCD1 gene could be as a possible predispose gene for SLE [28]. The interaction between PD-1 and its ligands (PD-L) reduces and negatively co-stimulates the auto-reactive T-cell and B-cell by the reducing the production of cytokine[29]. Therefore, impaired PD-1:PD-L function is implicated in several autoimmune disorders, including SLE, according to mounting evidence [30]. Patients with SLE had a significantly rise in levels of PD-1 expression on some cells, such as NK cells and both CD3 T and CD19 B lymphocytes [31]. The higher PD-1gene expression in patients with SLE is possibly illustrated by that PD-1 may play a role in SLE pathogenesis by other ways than its inhibitory function as a negative costimulatory molecule, it may also be linked to SLE severity and act as a negative feedback mechanisms, for the prevention of the potential tissue damage brought on the excessive autoimmune response in those with SLE [32]. Also, mutations that impair PD-1 regulation which have been linked to SLE in humans may occur[33]. The findings show a risen PD-1 expression in SLE patients may be related to the disease pathogenesis and may be helpful in SLE treatment. The current results supports Bassiouni et al [34] and Jiao et al., [27]. They discovered that compared to the healthy control, SLE patients' levels of PD-1 expression were considerably higher. While the present study contradicts Nishimura et al., [35] which discovered a lupus-like condition in animals lacking the PD-1 protein.

NFATc1 gene expression

Three of the four calcium-regulated NFAT proteins, including NFATc1, are expressed by T cells and are important regulators of T-cell activation, differentiation, and development [36]. NFATc1 has a high expression level in T cell [23]. The higher level of NFATc1 gene expression in those having SLE is explained through that, NFATc1 controls, among many other important genes, epression diverse proinflammatory cytokines and proteins inclduing IFN-γ, IL-4, and IL-17, which is highly involved in the SLE pathogenesis [37,38]. According to the study's findings, NFATc1 plays a part in the pathogenesis and severity of the disease. By focusing on NFATc1, a new SLE immunotherapy may be developed.

CONCLUSION

Based on these results, PD-1 and NFATc1 is important in the SLE pathogenesis. These results may hold promise for developing a new SLE immunotherapy by focusing on PD-1 and NFATc1.

REFRENCES

1. Kamil MA, Kadr ZHM, Alabassi HM. Role of CXCL9-CXCR3 AXIS, ANA & DS-DNA ABS in Pathogenicity of SLE in Iraqi Patients. Pak J Med Health Sci 2022; 16: 398-398.

2. Shedid NH., Hafez EA., Akram SA, et al. Ultrasonographic abnormalities of wrist and metacarpophalangeal joints in a cohort of Egyptian patients with systemic lupus erythematosus. Egypt. J. Intern. Med. 2019; 31:951-957
3. Abdul-Majeed NG. Antibodies to selected minor target anti-neutrophil cytoplasmic antigens in systemic lupus patients. J Fac Med Baghdad 2015; 57:231-235.
4. Lisnevskaia L, Murphy G, Isenberg d. Systemic lupus erythematosus. Lancet 2014;384:1878-88.
5. Abbas AH, Melconian AK, Ad'hiah AH. Role of Helicobacter pylori infection in etiology of systemic lupus erythematosus. Curr Res Microbiol Biotechnol 2017; 5:1285-1288.
6. Abdualkader SN, Al-Shaikly AW, Al-Mousawy KM, et al. Correlation between Interleukin-4 and Interleukin-6 and auto antibodies in Systemic Lupus Erythematosus. J Fac Med Baghdad 2009;51:416-418.
7. Abdulridha RH, Saud AM, Alosami MH. Evaluation of Interferon Alpha (IFN-α) in Women with Systemic Lupus Erythematosus in Iraq. Iraqi J Sci 2022; 63:4225-4233.
8. Al-Hammamy HR, Abd KH, Khalil IA, Mahdi BM. Human Leukocyte Antigens Assosiation with Systemic Lupus Arythematosus In Iraqi Patients. Al-Kindy Col Med J 2012; 8:84-88.
9. Pego-Reigosa JM, Nicholson L, Pooley N, et al. The risk of infections in adult patients with systemic lupus erythematosus: systematic review and meta-analysis. Rheumatology 2021; 60:60-72.
10. Kusnanto K, Sari NP, Harmayetty H, et al. Self-care model application to improve self-care agency, self-care activities, and quality of life in patients with systemic lupus erythematosus. J Taibah Univ Medical Sci 2018; 13(5):472-478.
11. Fife BT, Pauken KE. The role of the PD-1 pathway in autoimmunity and peripheral tolerance. Ann NY Acad Sci 2011; 1217:45-59.
12. Jubel JM, Barbati ZR, Burger C, et al. The role of PD-1 in acute and chronic infection. Front Immunol 2020; 11:487.
13. Al-Ammiri HH, Mohammed MM., Hussein AA. et al. Validity of Check Point Inhibitor (PD-1 and PD-L1) in Diagnosis of Gastric Adenocarcinoma Using Modified Tissue Elisa. Pakistan J. Medical Health Sci 2022;16:622-622
14. Deng Y, Huang F, Wang J. A narrative review of PD-1 and autoimmune diseases. Ann Blood 2022; 7:1-8.
15. Curran CS, Gupta S, Sanz I, Sharon E. PD-1 immunobiology in systemic lupus erythematosus. J Autoimmun 2019; 97:1-9.
16. Lee YH, Woo JH, Choi SJ, et al. Association of programmed cell death 1 polymorphisms and systemic lupus erythematosus: a meta-analysis. Lupus 2009;18:9-15.
17. Michot JM, Bigenwald C, Champiat S, et al. Immune-related adverse events with immune checkpoint blockade: a comprehensive review. Eur J Cancer 2016; 54:139-148.
18. Kuhn A, Bonsmann G, Anders HJ, et al. The diagnosis and treatment of systemic lupus erythematosus. Dtsch Arztebl Int 2015;112:423-432.
19. Prokunina L, Castillejo-López C, Öberg F, et al. A regulatory polymorphism in PDCD1 is associated with susceptibility to systemic lupus erythematosus in humans. Nat Genet 2002;32:666-669.
20. Reppert S, Zinser E, Holzinger C, et al. NFATc1 deficiency in T cells protects mice from experimental autoimmune encephalomyelitis. Eur J Immunol 2015;45:1426-1440.
21. Robbs BK, Cruz AL, Werneck MB, et al. Dual roles for NFAT transcription factor genes as oncogenes and tumor suppressors. Mol Cell Biol 2008; 28:7168-7181.
22. Rengarajan J, Tang B, Glimcher LH. NFATc2 and NFATc3 regulate TH2 differentiation and modulate TCR-responsiveness of naive TH cells. Nat Immunol 2002; 3:48-54.
23. Lee JU, Kim LK, Choi JM. Revisiting the concept of targeting NFAT to control T cell immunity and autoimmune diseases. Front Immunol 2018; 9:2747.
24. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1997;40:1725-1725.
25. Mosaferi E, Gharamaleki NA, Farzadi L, et al. The study of HLA-G gene and protein expression in patients with recurrent miscarriage. Adv Pharm Bull 2019; 9:70-75.
26. Ghorbani P, Mollaei H, Arabzedeh S, et al. Upregulation of single nucleotide polymorphism of PD-1 gene (rs10204525) in chronic hepatitis B patients. Int Arch Med Microbiol 2019; 2:1-8.
27. Jiao Q, Liu C, Yang Z, et al. Upregulated PD-1 expression is associated with the development of systemic lupus erythematosus, but not the PD-1.1 allele of the PDCD1 gene. Int J Genom 2014; 2014:1-6
28. Chua KH, Lian LH, Sim XJ, Cheah TE, Lau TP. Association between PDCD1 gene polymorphisms and risk of systemic lupus erythematosus in three main ethnic groups of the Malaysian population. Int J Mol Sci 2015; 16:9794-9803.
29. Carter LL, Fouser LA, Jussif J, et al. PD-1: PD-L inhibitory pathway affects both CD4+ and CD8+ T cells and is overcome by IL-2. Eur J Immunol 2002; 32:634-643.
30. Zamani MR, Aslani S, Salmaninejad A, et al. PD-1/PD-L and autoimmunity: a growing

relationship. Cell Immunol 2016; 310:27-41.
31. Eissa E, Kandil R, El-Ghobashy N, et al. Association of disease activity with programmed cell death 1 and its ligand programmed cell death ligand 1 expressions in lupus patients. Indian J Rheumatol 2022; 17: 347-352.
32. Luo Q, Kong Y, Fu B, et al. Increased TIM-3+ PD-1+ NK cells are associated with the disease activity and severity of systemic lupus erythematosus. Clin Exp Med 2022; 22:47-56.
33. Bryan CM, Rocklin GJ, Bick MJ, et al. Computational design of a synthetic PD-1 agonist. Proc Natl Acad Sci 2021;118:1-9.
34. Bassiouni SA, Abdeen HM, Morsi HK, et al. Programmed death 1 (PD-1) serum level and gene expression in recent onset systemic lupus erythematosus patients. Egypt Rheumatol 2021;43:213-218.
35. Nishimura H, Nose M, Hiai H, et al. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. Immunity 1999;11:141-151.
36. Macian F. NFAT proteins: key regulators of T-cell development and function. Nat Rev Immunol 2005;5: 472-484.
37. Xiao Y, Qureischi M, Dietz L, Vaeth M, Vallabhapurapu SD, Klein-Hessling S, et al. Lack of NFATc1 SUMOylation prevents autoimmunity and alloreactivity. J Exp Med 2021; 218:1-22.
38. Rafael-Vidal C, Altabás I, Pérez N, et al. Calcineurin and systemic lupus erythematosus: the rationale for using calcineurin inhibitors in the treatment of lupus nephritis. Int J Mol Sci 2021; 22:1263.

J Popul Ther Clin Pharmacol Vol 30(5):e260–e266; 05 March 2023.