Molecular effects of bone marrow derived-Mesenchymal stem cells (BM-MSCs) on some the cytokines encoding-genes in BALB/c mice with systemic lupus erythematosus (SLE)

Ghassan Khudhair Esmael[1,2*],
[1] *Department of Life Sciences, Faculty of Sciences-Sfax, University of Sfax, Tunisia*
[2]*University of Al-Qadisiyah, veterinary medicine college – Iraq*
*Corresponding author: **Esmael** ghassan.khudhair@qu.edu.iq

Tarek Rebai[3]
[3] *Laboratory Histo-embryology and Cytogenetics, Faculty of Medicine of Sfax, University of Sfax, Tunisia*

ABSTRACT

Lupus is a chronic autoimmune disease that occurs due to immune disturbance and poor regulation of the immune response, such as dysfunction of antibodies, immune cell, and cytokines. The study investigates the gene expression of some encoding chemokines and cytokines gene in BALB/c model mice with lupus. Forty male BALB/c mice are divided into four groups; each one consists of ten BALB/c mice. The first groups are injected activated lymphocytes Derivative DNA S/C for induction lupus. The second group was administrated activated lymphocytes Derivative DNA S/C for induction lupus then, treated with bone marrow derived-Mesenchymal stem cells (BM-MSCs); the third group was administered BM-MSCs intravenously only. The control group administrated PBS. After finishing the induction and treatment time, all the animals activated for take the serum for estimation the gene expression of the some of the cytokines and chemokines genes by using RT-PCR techniques in all the study groups. The findings are included that the first group showed significant high expression of IL-6, CCL-5/RANTES, VEGF, ICAM, CCL-2, and IFNγ-encoding genes; and low expression of IL-10 and TGFβ1-encoding gene as compared with the control group. The second group showed a significant low expression of IL-6, CCL-5/RANTES, VEGF, ICAM, CCL-2/MCP-1, and IFNγ-encoding genes while showing significant high expression of IL-10 and TGFβ1-encoding gene as compared with the control group. The third group doesn't show any changes in the studied cytokines and chemokines gene expression compared to the control group. BM-MSCs can effect on the gene expression of many cytokines and chemokine encoding genes, which treats induced lupus in mice.

Keywords: Mesenchymal stem cells, gene expression, cytokines genes, BALB/c mice, lupus

Introduction: lupus is a chronic immune disorder that affects humans and animals. Mesenchymal stem cells (MSCs) are adult stem cells; they can differentiate into several cell type lineages (1). MSCs have immunomodulatory impacts because they inhibit Th17 cells and stimuli Treg cells in autoimmune diseases (2).

MSCs can regulate the immunological response in vivo and in vitro in humans and mice by two mechanisms: forming of the soluble factors (IL-10, PGE2, and TGF-β1); and by cellular communication (3) (4). T helper cells are regulating the immune responses by producing cytokines (5). MSCs have an immunosuppressive effect; therefore, it used to treat lupus in the murine (6).

Treg cells have an important role in maintaining tolerance. Treg cells inhibit immune response, which has benefits for the treatment of lupus (7). MSCs showed that they are important in cases of Treg cells deficiency (8). MSCs improve the generation of Treg cells (9); therefore, it could be MSC to treat autoimmune diseases (10).

TGF-β1 is stimuli Treg cells (11). IL-10 and TGF-β1 increase the gene expression of FoxP3 in Treg cells. Differentiation of the Th cell inhibits differentiation of the Th17 cell, which regulate by IDO and PGE2 (12). TGF-β1 suppresses Th1 and Th2 cells, also Treg and Th17 cell induction (13-15). Th cells affect the regulation of cytokines and encoding genes (16).

TGF-β1 can inhibit Th1 and Th2 cells in autoimmune diseases such as lupus (17-19). TGF-β1 acts as an anti-inflammatory cytokine that has been used for the treatment of autoimmune disease (20). The current study investigates the effects of BM-MSCs on the gene expression of some chemokines-encoding genes and cytokines–encoding genes in BALB/C model mice with lupus.

Materials and methods:

The experimental animals: Forty BALB/c model mice provided at (6) weeks old with (20-30) grams purchased from (Jackson Laboratory, USA). It was housed in a pathogen-free animal house and under the same conditions.

The study design: Forty BALB/c mice are categories to (4) groups; each group consists of ten mice; the first group is administrated (ALD DNA) (50 µg/mouse) (ALD DNA was previous preparation) in three doses between one and another two weeks (0, 14, 28) days for inducing SLE in BALB/c mice. The second group is also administered (ALD-DNA) (50 µg/mouse), three doses at (0, 14, 28) days for inducing SLE; after the onset of the clinical signs, ANA and anti-dsDNA are examined for final diagnosis of SLE. The positive cases of SLE in the second group are treated by BALB/c-MSCs (CellBiologics company, USA) (0.1×10^6) cells/for 10g/IV. The third group is administrated BALB/c-MSCs only (0.1×10^6) cells/for 10g/IV. The fourth group (control group) administrated BPs only.

SLE induction: After receiving the shipped animals, some of the exported animals out of the experimental animals were used for the preparation of activated lymphocyte DNA (ALD DNA) according to (21), as three steps: Preparation of splenocytes from BALB/c mice spleens (22) (23), DNA extraction extracted from activated splenocytes. The animal was immunized with activated ALD DNA in three doses. The clinical signs appear after the third dose. Eliza kits did ANA and anti-dsDNA Examination.

The BALB/c BM-MSCs: The cells are provided by (Cell biologics Company, USA). It was administrated for the second and third groups at a dose (0.1×10^6) Cells (IV) for 10 g intravenous.

The used primers for gene expression: The tested genes are IL-10, IL-6, CCL-2, TGFβ1, IFNγ, CCL-5, ICAM, and VEGF genes in mice by using designed primers (depending on the genes database of NCBI (https://www.ncbi.nlm.nih.gov), and the primers are designed by primers 3 plus (https://www.primer3plus.com) in RT-PCR as shown in table (1)

Table (1): showed the used primers in the study

Gene	Product size	Direction	Seq.
IL-10	**242 bp**	**L**	**TCAGAGCTCCTGGAACTGGT**
		R	**TGCTAGAGCCCGGAGTTAAA**
IL-6	**185 bp**	**L**	**TTTCTCCACGCAGGAGACTT**
		R	**TCCACGATTTCCCAGAGAAC**
TGF-β1	**160 bp**	**L**	**ATTTTAGGGTGGCCCATTTC**
		R	**GAACTGACCCTGCTTCTTGC**

CCL-2/MCP-1	**200 bp**	**L**	**CCCAATGAGTAGGCTGGAGA**
		R	**GAACTGCCTTTGCCTTCTTG**
CCL-5/RANTES	**197 bp**	**L**	**GTGCCCACGTCAAGGAGTAT**
		R	**CTGGAGACGAGGAGCCATAG**
VEGF-A	**199 bp**	**L**	**ACCCGTGACTGAGGTTTGAC**
		R	**TTTCTTGCGCTTTCGTTTTT**
ICAM-1	**160 bp**	**L**	**AGCACCTCCCCACCTACTTT**
		R	**AGCTTGCACGACCCTTCTAA**
IFN-γ	**181 bp**	**L**	**ACTTTGCTTCTGCCTTTCCA**
		R	**ACAAGGTCACCCACAGGAAG**

Total RNA Extraction and Reverse Transcriptase:

RNA was extracted from animals serum directly by using RNA Extraction Kit (Addbio/Korea), 3). Extracted RNA was converted to cDNA by SYBR Green Master (Quantabio/Germany). The final volume 25 µl that consist of (SYBR Green Master Mix 12.5 µl, F primer and R primer 1 µl, DNA free water 6.5 µl, RNA template 3 µl and qScript One- Step RT3 1 µl). The total reaction mixture was incubated into the thermocycler. cDNA synthesis (49) ºC for (10) minutes., Taq activation 95ºC for (2) minutes., PCR cycling (38 cycles, 95 ºC for (4) seconds, and 59 ºC for (40) seconds)

The fold change are calculated as

2-ΔΔCTΔΔCT =ΔCT

ΔCT-ΔCT =CT gene-CT House Keeping gene.

Statistical Analysis: The results were analyzed by an ANOVA test (SPSS, V20; USA). Using of LSD test to determine the difference among the groups at P= 0.05 (24).

Results:

The gene expression of the IL-10 gene in G1 was low than in control. No significant differences exist among other groups in gene expression of the IL-10 gene, as shown in table (2) and figure (1).

Table (2): Values of the fold change of the IL-10 gene

Group	Fold change
G1	0.223 B
G2	0.905 A
G3	0.998 A
G4	1 A

The same letters haven't significant differences; the different letters have significant differences

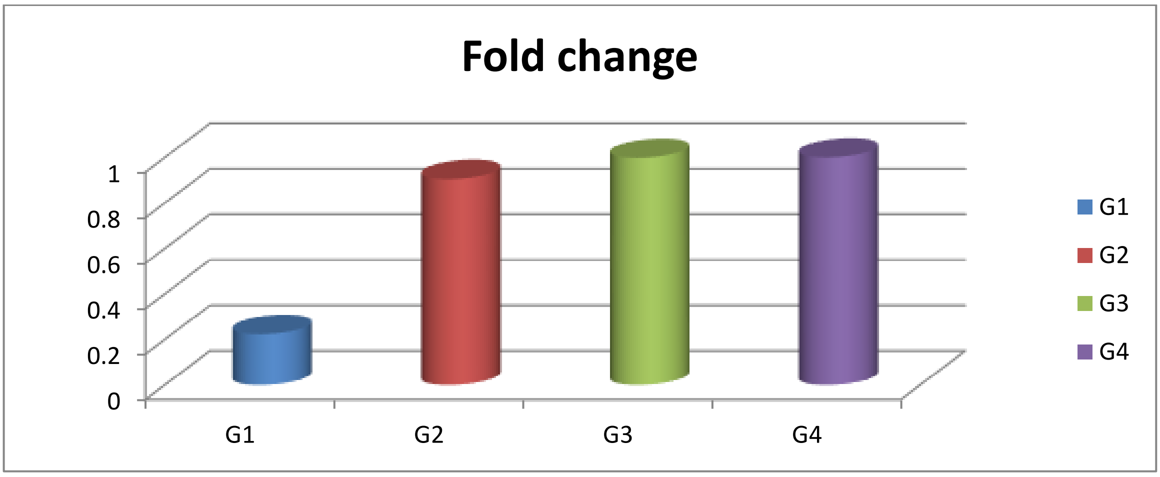

Figure (1): the chart shows the fold changes of the IL-10 gene

The current study showed that the IL-6 encoding gene expression was increased in G1 than in the control. There are no significant differences among the other groups in gene expression of the IL-6 gene, as shown in table (3) and figure (2).

Table (3): Values of the fold change of IL-6 encoding gene

Group	Fold change
G1	1.885 A
G2	1.051 B
G3	0.997 B
G4	1 B

The same letters haven't significant differences; the different letters have significant differences

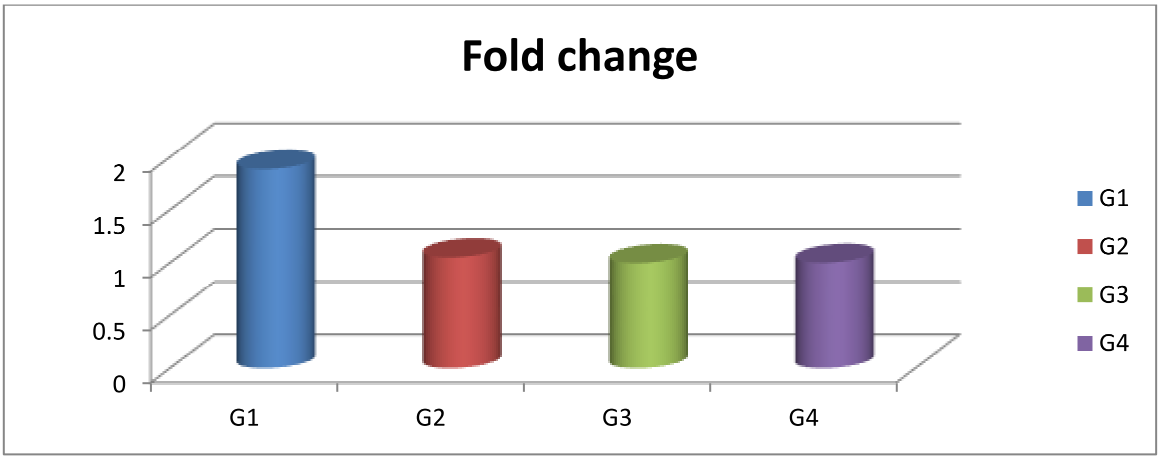

Figure (2): chart shows the fold changes of the IL-6 gene

The findings showed that the TGF-β1 expression gene was reduced in G1 than in the control. There are no marked differences among other groups in the TGF-β1 expression gene, as shown in table (4) and figure (3).

Table (4): The fold change values of the TGF-β1 gene

Group	Fold change
G1	0.365 A
G2	0.996 B
G3	0.997 B
G4	1 B

The same letters haven't significant differences; the different letters have significant differences

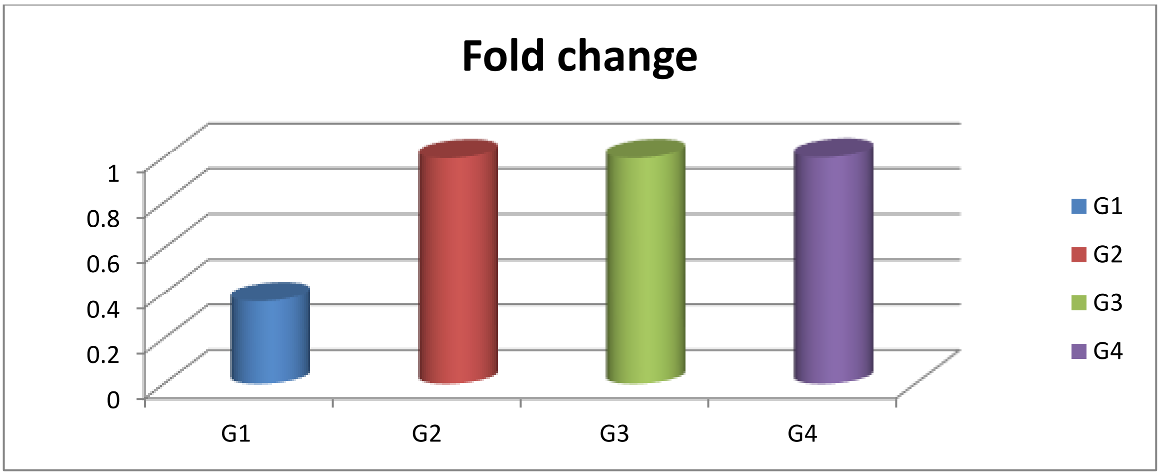

Figure (3): chart shows the fold changes of the TGF-β1 gene

The current study showed that the gene expression of the CCL-2/MCP-1 encoding gene was increased in G1 than in the control. There are no marked differences among other groups in gene expression of the CCL-2/MCP-1-encoding gene, as shown in table (5) and figure (4).

Table (5): The fold change values of the CCL-2/MCP-1 gene

Group	Fold change
G1	3.299 A
G2	1.0544 B
G3	0.97368 B
G4	1 B

The same letters haven't significant differences; the different letters have significant differences

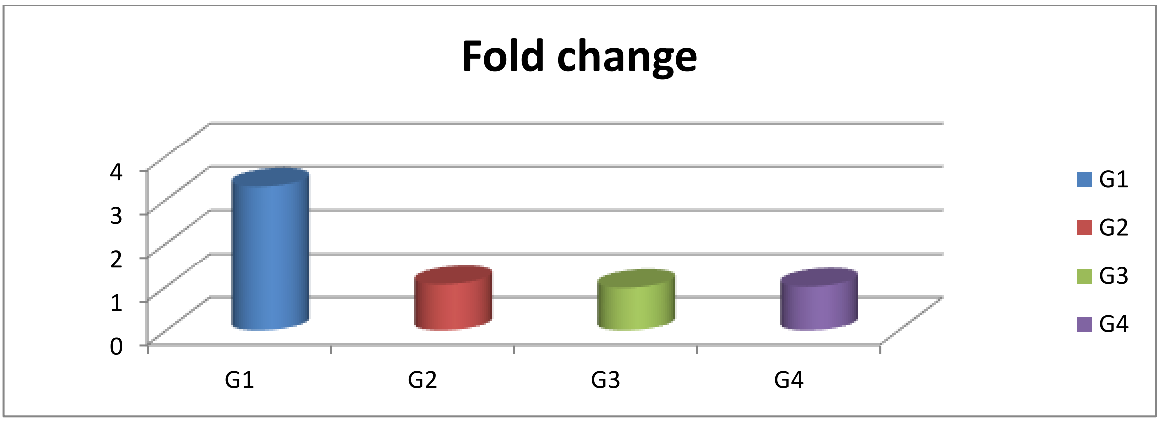

Figure (4): chart shows the fold changes of the CCL-2/MCP-1 gene

The current study showed that the gene expression of the CCL-5/RANTES-encoding gene was increased in G1 than in the control. There are no marked differences among other groups in gene expression of the CCL-5/RANTES-encoding gene, as shown in table (6) and figure (5).

Table (6): Values of the fold change of CCL-5/RANTES encoding gene

Group	Fold change
G1	5.48684 A
G2	0.96846 B
G3	1.00919 B
G4	1 B

The same letters haven't significant differences; the different letters have significant differences

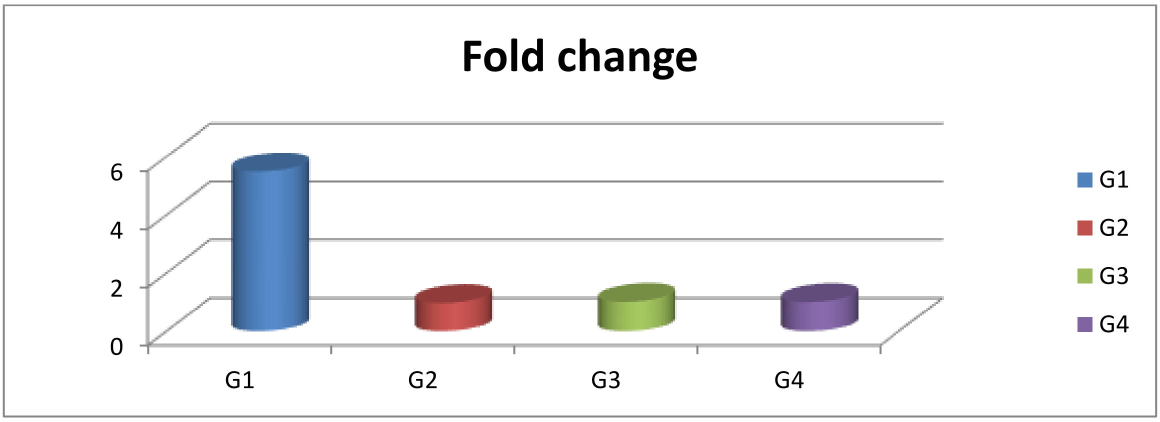

Figure (5): chart shows the fold changes of the CCL-5 gene

The gene expression of the VEGF-A encoding gene was increased in G1 than in the control. There are no marked differences among other groups in gene expression of the VEGF-A-encoding gene, as shown in table (7) and figure (6).

Table (7): Values of the fold change of the VEGF-A encoding gene

Group	Fold change
G1	4.30452 A
G2	1.134773 B
G3	0.990740 B
G4	1 B

The same letters haven't significant differences; the different letters have significant differences

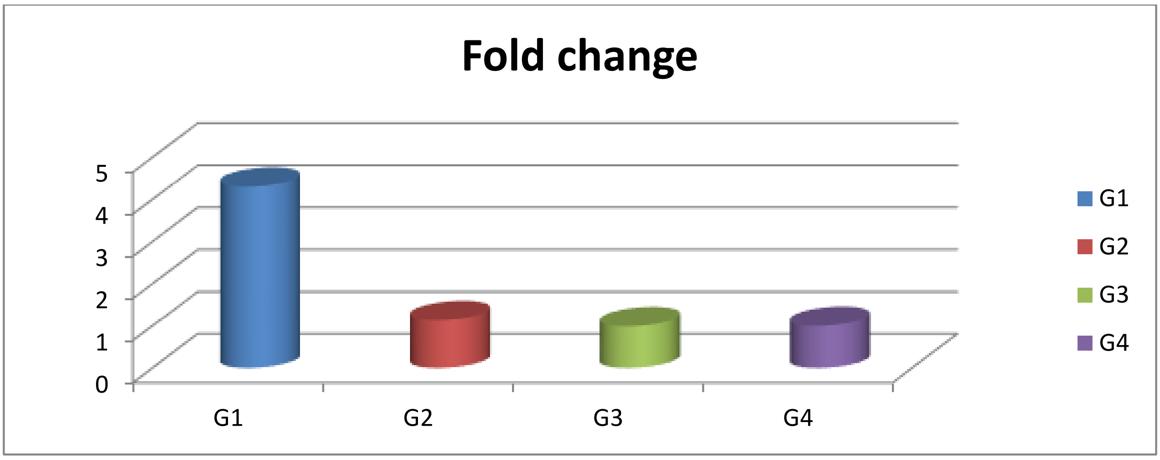

Figure (6): chart shows the fold changes of the VEGF-A gene

The current study showed that the ICAM-1 encoding gene expression was increased in G1 than in the control. There are no marked differences among other groups in gene expression of the ICAM-1-encoding gene, as shown in table (8) and figure (7).

Table (8): Values of the fold change of the ICAM-1 gene in study groups

Group	Fold change
G1	1.34562 A
G2	1.00755 B
G3	1.000888 B
G4	1 B

The same letters haven't significant differences; the different letters have significant differences

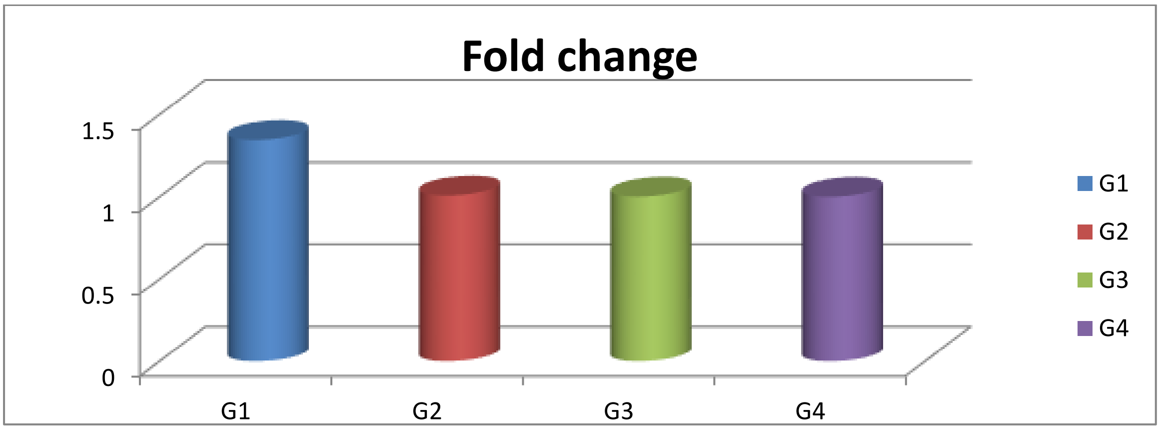

Figure (7): chart shows the fold changes of ICAM-1 gene in study groups

The current study showed that gene expression of the IFN-γ-encoding gene was increased in G1 than in the control. There are no marked differences among other groups in gene expression of the IFN-γ encoding gene, as shown in table (9) and figure (8).

Table (9): Values of the fold change of IFN-γ-encoding gene in study groups

Group	Fold change
G1	2.135330 A
G2	0.992939 C
G3	0.994731 B
G4	1 B

The same letters haven't significant differences; the different letters have significant differences

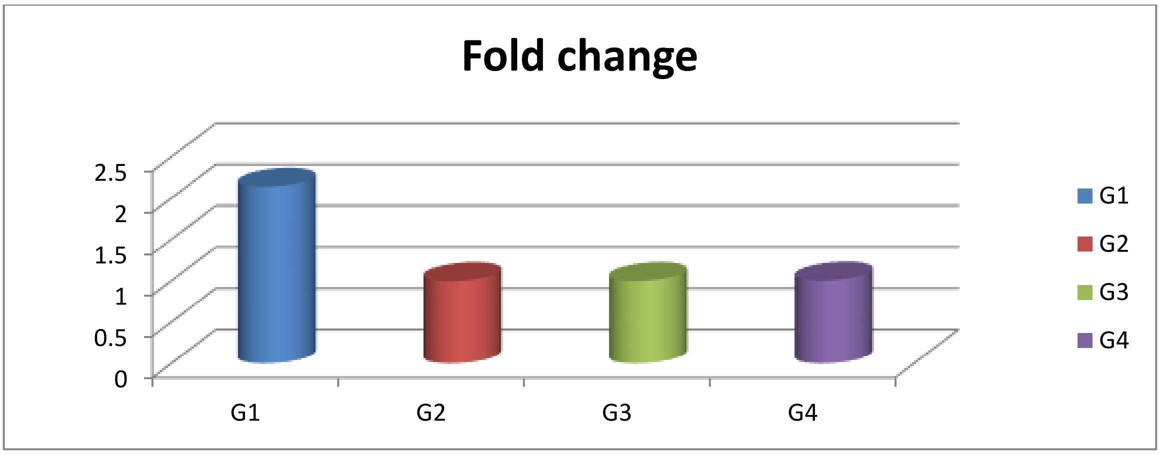

Figure (8): chart showed the fold changes of the IFN-γ gene in study groups

Discussion:

Lupus is a complex autoimmune disease; several medicine drugs could be used to treat lupus, such as Methotrexate, Mycophenolate mofetil, Azathioprine, Cyclophosphamide, and Voclosporin (24). In the last decade, many researchers found that body tissues derived-Mesenchymal stem cells are important in treating lupus. Mesenchymal stem cells reduce the pro-inflammatory cytokines and increase anti-inflammatory cytokines by producing several factors such as exosomes, microvesicles, apoptotic bodies, proteins, and lipids. These factors play a crucial role in regulating immune responses by control on gene expression (25) (26).

Our findings included high gene expression of IL-6, CCL-5/RANTES, VEGF, ICAM, CCL-2, and IFNγ-encoding genes; and a decrease in gene expression of IL-10 and TGFβ1 gene in the first group. The second group showed low gene expression of IL-6, CCL-5/RANTES, VEGF, ICAM, CCL-2/MCP-1, and IFNγ while showing significantly high gene expression of IL-10 and TGFβ1 gene than in the control group. MSCs are used to treat autoimmune diseases by regulating the gene expression of cytokine-encoding genes (27) (28). The low expression of TGF-β1 is associated with the common expression of IL-10 in lupus. Lupus showed a deficit in Treg cells which can treat by MSCs. MSCs reregulate the immunological response, increase Treg cell counts in lupus cases, and increase expression of TGF-β1 and IL-10 to maintain immune activity (29). TGF-β1 was decreased in the patients with SLE than in the control group (30).

TGF-β1 and IL-10 expression was increased in lupus patients compared to the control group. TGF-β1 and IL-10 are increased in lupus patients. IL-10 and TGF-β1 inhibit the autoreactive immune cells in lupus patients (31). mRNA expression of IL-6 and TNF-α were decreased in MSCs in Osteoarthritis cases than in the control group using RT-PCR. Furthermore, the mRNA concentration of IL-10, IL-4, and TGF-β1 in MSCs was increased than in the control group, which showed agreement with our results (32).

Treatment by MSCs was used in regeneration medicine and autoimmune diseases. MSCs can migrate to injured sites for differentiation to produce many bioactive factors, such as cytokines and growth factors, which provide microenvironment regeneration and inhibit local inflammation. The differentiation of MSCs is associated with cytokines and proteins. MSCs stimulate the anti-inflammatory cytokine by regulating the expression of the cytokines encoding genes. TGF-β1 gene expression was increased than the control group. MSCs could regulate the gene expression of the cytokines genes (33). Gene expression of the cytokines was changed during the MSCs differentiation (34).

IL-6 was down-regulated in lupus patients after treatment by MSc. The impaired MSCs to secrete the immune factors are attributed to the genetic disorder of lupus cases due to weakened MSC. IL-6 decreased MSC, while TNF-α increased MSC activity by stimulating IDO in MSCs (35) (36). Interferon-γ and IL-6 expression were reduced in T lymphocytes of lupus patients with MSCs (37). MSCs were stimuli to the regulatory B cells in the mice with the autoimmune disease. MSCs inhibit anti-dsDNA and regulate imbalances among Treg/Th17/Th1 (29). Many reports found a relationship between the effects of t MSCs on the gene expression of the cytokines wherever; MSCs can treat collagen-induced arthritis through the modulation of the expression of the cytokines (38).

MSCs have a potent immunosuppressive effect in vivo (39). MSCs can decrease interleukin 10 concentration in pulmonary vasculitis in lupus mice. MSCs are strong immunomodulatory cells investigated in graft versus host disease, SLE, Crohn's disease, and ulcerative colitis (40). MSCs have an immunosuppressive effect on the immune system (41). MSCs have distinct gene expression (42). MSc has an immunomodulatory role in autoimmune disease (43). The genetic exchange between resident and MSCs by the microvesicles showed instrumental in MSc therapeutic effects (44). The cytokine produced by MSc has an important role in autoimmunological disorders such as SLE (45).

MSc transplantation has important therapeutic effects on treating rheumatoid arthritis, SLE, Crohn's disease, juvenile idiopathic arthritis, systemic sclerosis, multiple sclerosis, and type I diabetes mellitus (46). MSc has expressed the surface markers and secreted factors. MSc genes play an important role in therapeutic outcomes (47). MSc contributes to the immunosuppressive in persistent apical periodontitis (48). All the above and mentioned studies agreed with our study findings and directly supported our results.

Conclusion: BM-MSCs can effect on the gene expression of some cytokines genes in BALB/C mice with lupus.

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