Farhan A.Risan /J. Pharm. Sci. & Res. Vol. 10(4), 2018, 860-862

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Detection of interleukin-17 A and interferon –gamma concentrations in systemic Lupus erythrmatosus patients in Baghdad

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Abstract :

This study was carried out the period September/2016 to December 2017,for measurement the concentration of interleukin 17-A and interferon-gamma in the sera of systemic lupus erythrmatosus(SLE) patients (37 patients ,34 female & 3 male) compared with (37 healthy persons, 34 female & 3 male) as control group.

The diagnosis of SLE patients were done by enzyme linkage immunosorbent assay (ELISA) . A high significant increment ($P< 0.01$) was observed in the sera of SLE patients (34.72 pg/mL) compared with healthy control (26.19 pg/mL) in IL-17,while (71.99pg/mL) in the patients of SLE compared with healthy control (49.32 pg/mL) in interferon gamma .The laboratory features haemoglobin concentration ,erythrocyte sedimentation rate, white blood cells, platelets and albumin were done , all these tests showed high significant differences ($P< 0.05$,$P < 0.01$).The anti ds-DNA was done to all SLE patients.

Key words : Systemic Lupus erythrmatosus ,Interleukin -17A,Interferon gamma

INTRODUCTION :

Systemic Lupus erythrematosus (SLE) is a multisystem involvement and autoantibodies to nuclear , cytoplasmic and cell surface auto-antigens(1,2).The cause of SLE is as yet unknown and the pathogenesis is more complex involves immunological, hormonal, hereditary and environmental factors and the disease shows a striking in female and affects predominantly women in their reproductive ages ,the prevalence varies between ethnic groups 1:250 in black women and 1:4300 in white women (3,4).

In SLE disease representative overproduction of autoantibodies, deposition of immune complexes and hyperactivity of both T&B lymphocytes resulting sever damage to multiple organs and connective tissue in large population through the world (5).

SLE is heterogenous autoimmune disorder with severe clinical manifestation from the skin lesion to multisystem dysfunction such as:respiratory distress, pericarditis, osteoporosis and renal failure (6).

Cytokines play a crucial role in the production ,maturation and differentiation of immune cells, the effect of cytokines in autoimmune SLE cannot be consistently predicted ,these effects are much more complex than the simplistic(7).

Interleukin 17 A is a multifunctional cytokine that impacts neutrophil recruitment,mediating both T-helper-1 (Th1) and T-helper -2 (Th2) cytokine production, and it possesses angiogenic properties through apoptosis (8,9,10).

In humans IL-17A is primarily expressed by the T-helper-17 (Th17) subset of CD4 T cells(11),but is also produced by neutrophils,natural killer (NK) cells and CD8+ and double negative (DN) T cells (8).

IL-17 production *in vivo* and *in vitro* is primarily controlled by transforming growth factor beta-1(TGF-B1) and interleukin-6 (IL-6) via the activation of signal tranducer and activator of transcription-3 (STAT-3) in mouse and human models respectively (12,13).

Recent reports of IL-17 inhibition in achieving clinical benefit in ankylosing spondylitis, questions will be raised regarding to the potential to exploit IL-17 inhibition in SLE patients or certain symptoms groups (14,15).

Interferon is water-soluble dimer cytokine in the only member of type –II interferon initially called macrophage activating factor.The biologically active INF-y is formed by two anti-parrel interblocking monomers ,which is composed of six alpha helices consisting of one core and a fragment sequence extending at the C-terminal region . IFN-y is a typical kind of cytokine secreted by Th1 cell and its expression profile is regulated by a series of IFN inducing factors including IL-12,15,18 (16,17).

IFN-y is initiated in the early stages of the innate immune response and is argued to amplify the adaptive autoimmune response, it has been suggested that it plays the dominant role (18).

IFNs are a family of proteins consisting of three major types ;type 1(IFN-α,β),type II INF-y and type III IFN-γ1, IFN-γ2, IFN-γ3,these three types differ in their primary protein sequences, congate receptors,chromosomal locations and the types of cells responsible for production of response , the interferon influence wide variety of biological responses including providing from viral and bacterial infections, antitumor effects and regulation of effector cells in both innate and adaptive immune responses(19,20).

Therefore the aim of this study was to investigate IL-17 A and IFN- γ levels and serological association between SLE patients and healthy control.

MATERIALS AND METHODS

Study subjects : A total of 37 patients(34 female & 3 male) with SLE and 37(34 female & 3 male) as healthy control were enrolled for this study , patients were elected from the outpatients of nephrologists after clinical diagnosis, the laboratory tests were done as a rapid latex agglutination test for detection of the SLE in human sera (patients and control groups).Latex serology test by OMEGA diagnostic company ,UK,to detect and quantitative of antibodies in the sera to double strand dsDNA.

The Avitex SLE particles are coated with deoxyribonucleoprotein(DNP),when the latex suspension is mixed with serum containing anti-double strand of DNA antibodies,a clear agglutination is seen within 3 minutes.This test has been calibrated against the WHO.

The sera of patients were diluted by isotonic saline to 1/2 ,1/4, 1/8 , 1/16 ,1/32,1/64 ;50 µL of each diluted serum to the test circle and one drop of suspension to the test circle mixed well,the negative gives negative result after 3 min. . A positive gives positive result after 3 min. at a titer of 1/4 and more.The haemoglobin concentration ,white blood cells,erythrocyte sedimentation rate,platelets and albumin were done for all SLE patients.

Sample collection

Venous blood 5 milliliter without anticoagulant was drawn from the control and patients, the blood sample were allowed to 30 min.and centrifuged ,serum was separated and stored in deep freezer until the estimation of cytokines.

Estimation of IL-17 A

The level of IL-17 A in the sera were determined by using a solid phase enzyme linked immunosorbent assay (ELISA).The ELISA kit from Cusabio company,the assay procedure as follows:

1-All reagents were prepared,working standards and samples.

2-One hundred microliter of standard and samples per well wer added ,covered with the adhesive strip,incubate for 120 min. at 37°C.

3-Removed the liquid from each well.

4-One hundred microliter of biotin-antibody (1x) to each well, covered the microtiter plate with non adhesive strip ,incubated for one hour at 37°C.

5- Aspirate each well and wash 3 times.

6-Added 100 µL of HRP-avidin (1x) to each well,covered by new adhesive strip,incubate for 1 hour at 37°C.

7- Aspirate each well and washed 5 times.

8-Added 90 µL of TMB substrate to each well ,incubate at 37°C for 15-30 min.

9-Added 50 µL of stop solution to each well.

10-Read at 450 nm.

Estimation of IFN-γ

The levels of IFN-γ in the sera were determined by using a solid phase enzyme linked immunosorbent assay(ELISA).The ELISA kit from Cusabio company .The assay procedure as follows:

1-All reagents were prepared (working standards,and samples).

2-One hundred microliter of standard and samples per well added .Covered with adhesive strip,incubated for 120 min.at 37°C.

3-Removed the liquid from each well.

4-One hundred microliter of biotin-antibody(1x) to each well ,covered the microtiter plate with new adhesive strip,incubated for one hour at 37°C.

5- Aspirated each well and wash 3 times.

6-Added 100 µL of HRP –avidin (1x) to each well ,covered by new adhesive strip.Incubate for one hour at 37°C.

7-Aspirate each well and washed 5 times.

8-Added 90 µL of TMB substrate to each well ,incubated at 37°C for 15-30 min.

9-Added 50 µL of stop solution to each well.

10-Read at 450 nm.

The results were performed by statistical package for the social sciences (SPSS)

RESULTS

Table (1) shows the gender ,age and anti-ds DNA titer in the SLE patients compared with healthy control,there was no significant difference in the gender (male/female) and age of SLE patients and healthy control.The titer of anti- dsDNA in the SLE patients was 1/32 while negative in healthy control.

Table (1):Age ,gender and titer of ds-DNA in the sear of patients with SLE and healthy control

Variable	SLE patients	Healthy control	P.value
Gender(Male & Female)	3/34	3/34	N.S
Age (years ± S.D)	32.3 ± 7.8	35.6± 8.1	N.S
Anti-ds DNA titer	Positive Titer(1/32)	Negative (Not avariable)	Not avariable

Table (2) shows laboratory features of SLE patients of haemoglobin concentration,erythrocyte sedimentation rate,white blood cells,platelets and albumin.Shows the haemoglubin and albumin a significant differences at 0.05 in SLE patients compared with healthy control,while erythrocyte sedimentation rate ,platelets and white blood cells count show a high significant difference between SLE patients and healthy control ($p < 0.01$).

Table (2) :Laboratory investigation of SLE patients & healthy control

Parameter	SLE patients	Healthy control	P.value
Hb % g/L	12.3	13.8	0.05
ESR mm/hr	23	12	0.01
WBC	5,200	6400	0.01
PLT	234,000	251,000	0.01
Alb. g/L	3.9	4.7	0.05

Hb =Haemoglobin ,ESR=Erythrocyte sedimentation rate , WBC=White blood cells,PLT=Platelets,Alb=Albumin

Table (3) shows the concentration of IL-17 A & IFN-γ in the SLE patients & healthy control.There was highly significant difference (P 0.01),the level of IL-17 A in SLE patients 34.7pg/mL and in healthy control 26.19 pg/mL,while IFN-γ ,the level in SLE patients 71.99 pg/mL and healthy control 49.32 pg/mL

Table (3): Level of IL-17 A & IFN-γ in the sera of SLE patients & healthy control

Parameter	SLE patients	Healthy control	P.value
IL-17 A pg/mL	34.72 ± 5.99	26.19 ± 4.72	0.01
IFN-γ pg/mL	71.99 ± 9.83	49.32 ± 6.71	0.01

DISCUSSION

Increase the concentration of of IL-17 A in the sera of SLE patients probably contribute to the recruitment and activation of immune cells to target organs and thus amplify an ensuring immune response,the immune environment in patients with SLE is ideally suited for the generation of IL-17 A producing T-cells.

The high level of IL-17 A in the sera of SLE patients found by other researchers suggest in the pathogenesis of SLE,because these cytokines produced by helper T lymphocyte responsible for immune response ,which may be disregulated in the impaired immune response (22,23) . IL-17 consists of IL-A,B, and F and each cytokine can be composed of either homodimers or heterodimers ,these types of cytokines enhance the production of TNF,L-1, granulocyte colony-stimulating factor,chemokines and other molecules (24).In Lupus as in a number of other autoimmune disease,Th-17 cells traffic to inflamed tissue to promote inflammation by enhancing cytokine production which can in turn activate B cells antibody production ,activate dendritic cells ,and stimulate resident cells in the target tissue (25,26).

IFN-γ principally produced b y T-cells,CD4+ as well as CD8+ and natural killer cells ,links innate and acquired response of macrophage(27,28). IFN-γ signal promote systemic lupus via direct action on B cells mechanistically, IFN-γ promotes the formation of spontaneous autoimmune germinal centers via reciprocal activation of germinal center B cells and T follicular helper cells (29). The elevated level of concentration of IFN-γ in SLE patients agree with (30) reach to closely association between SLE pathogenesis and IFN-γ IFN-γ elevation.

IFN-γ activates macrophage microbicidal functions, up regulates class I ,and class II MHC molecules on antigen presenting cells , modulates T cell differentiation ,and is involved in B cell isotypes which to opsonizing and complement fixing antibody subclasses (31).The IFN-γ the most important cytokine in Th1 derived cytokines family ,it plays a key role in the differentiation of Th1 cells,which can specifically stimulate the signal transductor and activator of transcription -4 (STAT-4) and transcription factor ,thereby promote the differentiation of naïve T cells toward the Th1 phenotype whatever *in vivo* & *in vitro* (32,33) .

From present study we concluded the IL-17 A & IFN-γ participating in inflammation may also serve a protective purpose in SLE patients.

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