Original Article

Interleukin-22 and Tumor Necrosis Factor Receptor Associated Factor 1 (TRAF1) in Patients with Rheumatoid Arthritis and Systemic Lupus Erythematosus: Correlation with Disease Activity

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ABSTRACT

In autoimmune diseases including rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), cytokines play a central role in initiating and maintaining diverse immune and inflammatory responses. Interleukin 22 (IL-22), a member of IL-10 cytokine family, has been believed to be an important player in regulating inflammatory responses associated with many autoimmune diseases. The aim of this study was to detect the role of IL-22 and Tumor necrosis factor receptor associated factor 1 (TRAF1) in patients with RA and SLE and their correlation with disease activity. The current study was conducted on 70 RA, 64 SLE patients and 45control subjects from Egyptian population, using ELISA to assess IL-22 in culture supernatants of cultured lymphocytes and TaqMan genotyping assay for TRAF1 (rs10818488). A significant increase in IL-22 levels in both RA and SLE patients than control group ($p<0.001$ for each) was detected before and after lymphocyte stimulation. Also, a significant differences in A allele frequency with RA patients was found ($P=0.030$) indicating that TRAF1 could be considered as a susceptibility gene to RA in the Egyptian population. The A allele of TRAF1 was significantly increased RA patients with positive rheumatoid factor (RF) ($p=<0.001$) and/or anti-CCP antibodies ($p=0.034$), this could not be demonstrated in SLE patients or controls($P=0.750$). Also, IL-22 level correlated positively with disease activity in RA and SLE patients which may rise a possible role in the pathogenesis of both conditions. **[Egypt J Rheumatology & Clinical Immunology, 2016; 4(1): 49-58]**

Key Word: IL-22 ; TRAF1; systemic lupus erythematosus; rheumatoid arthritis

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune, systemic inflammatory disorder that causes an inflammatory response in the synovium (synovitis) leading to destruction of the articular cartilage and ankylosis of the joints. RA can also produce diffuse inflammation in the lungs, pericardium, pleura, and sclera.[1]

It affects 0.5-1% of general population with variations in the incidence and prevalence across different population, these variations occur as a result of differences in genetic composition and ethnicity.[2]

Systemic lupus erythematosus (SLE) is also a chronic autoimmune disease that causes multiple organ damage and is associated with the production of autoantibodies. Although the etiology and pathogenesis of SLE is not clear, it may be an immune regulation disorder caused by a complex interplay of genetic and environmental factors.[3]

In both diseases, cytokines play a fundamental role in the associated inflammation.[4] Typically, neutralization of the pro-inflammatory cytokines including tumor necrosis factor alpha (TNF-α) improves RA and SLE.[5] Nevertheless, there are some patients who do not respond to this therapy, possibly due to the genotypic background of TNF-α and TNF receptor (TNFR) genes that could account for patients' resistance to TNF-α blockers.[6]

TNF-α exerts its effects through type 1 (TNFR1) or type 2 (TNFR2) receptors but it is generally admitted that TNFR1 mediates most of TNF-α biological effects. Signaling occurs through two principal classes of cytoplasmic adaptor proteins: TNF-receptor associated factor (TRAF) and TNFR associated death domain (TRADD). TRAF family consists of six members that are involved in intracellular signal transduction. The TRAF1 region is immune-related gene that is involved in both the onset and perpetuation of inflammation.[7]

Genetic factors seem to play a key role in the susceptibility to SLE and RA. In the past several years, genome-wide association studies (GWAS) for SLE have identified literally hundreds of genetic loci involved in the susceptibility conferred to complex inherited traits.[8] Even though this scenario represents an extraordinary advance in complex disease genetics, the modest effect sizes of the common polymorphisms found associated explain only a small fraction of the heritability in most of these multifactorial conditions, suggesting that many more loci remain to be discovered.[9] A large-scale and candidate gene association studies have identified a number of Single nucleotide polymorphism (SNP) markers that are associated with RA and SLE susceptibility improving our understanding of the genetic component of disease susceptibility.[10] To have a clinical impact, these markers must be associated with prognosis and treatment. The gene encoding TRAF1 (rs10818488a) is located within a region coding for the transcription factor P300 which plays important roles in many biologic processes, including cell proliferation and differentiation and plays a key role in adaptive immunity of SLE and RA.[11]

However, other inflammatory pathways, which are potentially TNF α-independent, can as well contribute to drug resistance, indicating that there are other cytokines and growth factors, which may play important roles in the disease process.[12]

Th17 cells and their cytokines are associated with several autoimmune and inflammatory diseases, such as RA, SLE, multiple sclerosis (MS), psoriasis, inflammatory bowel disease (IBD), allergy and asthma.[13]

IL-17 operates on a variety of cell types and synergizes with TNF to promote inflammatory responses. IL-17 family members are important in controlling extracellular bacterial and fungal infections. Development of Th17 cells requires IL23 and IL-1b and other transcription factors. In addition to IL-17A and F, Th17 cells also secrete IL-21, IL-22, and granulocyte macrophage colony-stimulating factor (GM-CSF).[14]

Interleukin 22 (IL-22), a member of IL-10 cytokine family, exerts its effects via a heterodimeric transmembrane receptor complex.[15] In addition to Th17 cells, the major source of IL 22 is the newly discovered subset, Th22 cells that recently expanded the T helper cell family. Its characteristic functional profile is mediated by distinct cytokines, they secrete TNF-α and their lead cytokine IL-22 .IL-22 has been believed as an important player in regulating inflammatory responses associated with many inflammatory diseases.[16] However, paradoxical results of IL-22 serum sample levels from different chronic inflammatory diseases have been obtained in different studies that led to the belief that diverse pathogenic mechanisms and tissue microenvironments may result in different contributions of IL-22 in autoimmune disease development.[17]

There is apparently a strong functional synergism of TNF-α and IL-22[18]; the interaction of IL-22 and TNF-α is mediated through the tumor necrosis factor receptor I and IL-22R heterodimer and intracellularly by MAP kinases. The combination of IL-22 and TNF-α strongly induced the phosphorylation and translocation of MAP kinases to the nucleus whereas the single cytokine only weakly contributed to MAP kinase activation. The discovery of the IL-22/TNF-α axis gives a first insight that could lead to new therapeutic approaches of RA.[19]

Aim of the study:

The aim of the present study was to evaluate the role of IL-22 and TRAF1 in patients with RA and SLE, unraveling their association to the disease activity.

PATIENTS AND METHODS

The study included 70 patients affected by RA, 64 patients with SLE and 45 healthy age and sex matched controls. Patients were attending the outpatient clinic of Rheumatology department, Alexandria University Hospital between January 2015 and November 2015 and were enrolled into the study according to the ACR/ EULAR (American College of Rheumatology/ European League Against Rheumatism) classification criteria for RA[20] and 2012 revised SLICC criteria for SLE patients.[21] Before investigation, written informed consent was obtained from all participants approved by local ethics committee of Medical Research Institute, Alexandria University. Patients were chosen free from infectious or other autoimmune diseases that may interfere with results of the study.

Clinical Assessments

For RA patients, Disease activity score-28 (DAS28) was calculated according to the formula that is composed of the number of tender and swollen joints, patient's global assessment of disease activity on a visual analogue scale (VAS) and ESR.[22] Examined joints are: proximal interphalangeal joints (PIP), metacarpophalangeal joints (MCP) of both hands, both wrist, elbows, shoulders and knees. Predefined cut-offs for remission, mild, moderate and severe disease activity according to DAS 28 score are < 2.6, 2.6 - 3.2, > 3.2 - < 5.1 and > 5.1 respectively

For SLE patients, disease activity was scored using SLE Disease Activity Index (SLEDAI) to assess lupus activity.[23] SLE patients were arbitrarily

categorized into 2 groups: mild to moderate flare (SLEDAI score > 3) and severe flare (SLEDAI ≥ 12).

Sample Collection and Analysis

Ten ml of venous blood were drawn under complete aseptic technique. For detection of TRAF1 polymorphism, DNA was isolated and purified from whole blood (EDTA) using QIAamp - spin columns according to the protocol provided by the manufacturer (QIAamp DNA Blood Mini Kit).[24] Heparinized blood was used for Peripheral blood mononuclear cells isolation for IL-22 assay. In addition the separated serums were collected and stored in −80 °C until laboratory tests were done. The serum concentration of CRP and RF was measured by the BN Pro Spec Nephelometry. (Siemenes, USA). Anti-CCP was measured by enzyme linked immunosorbent assay (ELISA) kit (anti-CCP The Diagnostic Automation, Inc. >30 U/mL). The ESR was measured using Westergren method. Hemoglobin level, WBCs count and platelets count were analyzed by the H1-Technicon blood cell counter. IL-22 concentration was detected in culture supernatent by ELISA (WKEA Med Supplies).

Methods

DNA isolation

DNA was isolated and purified from whole blood (EDTA) using QIAamp - spin columns according to the protocol provided by the manufacturer (QIAamp DNA Blood Mini Kit). ([24])

Separation of lymphocytes

Peripheral blood mononuclear cells were isolated using Ficoll- Hypaque. Monocytes were allowed to adhere to cultured plated. Retrieved lymphocytes pellet was resuspended in complete culture medium composed of RPMI-1640 supplemented with 10% heat-inactivated fetal calf serum, 100 IU/ml penicillin and 100 µg/ml streptomycin and counted in a hemocytometer.[25].

Cell Culture

Lymphocytes adjusted to 2×10^6 cells/ml were maintained in a short-term (48 hours) culture for assessment of IL-22 with 100 µl of the supplemented RPMI-1640 tissue culture medium. Phytoheamagglutinin (PHA) and Anti-Human CD3 mAb [OKt 3; eBioscience, Cat. No. 14-0037-82] diluted in carbonate buffer (32 mM Na2CO3/16 mM $NaHCO_3$) from 100 ng/µl stock solutions directly were added to only one set of the cultures lymphocytes.

Detection of IL-22 in culture supernatant by ELISA

IL-22 concentration in culture supernatant of peripheral blood mononuclear cells (PBMCs) was determined using sandwich ELISA and measured spectrophotometrically at a wavelength of 450 nm. The concentration of IL-22 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Analysis of rs10818488 (TRAF1) polymorphism

Genotyping of SNP was carried out using the TaqMan genotyping assay (Applied Biosystems-Life Technologies, Carlsbad, California, USA), according to the manufacturer's instructions. Predesigned TaqMan SNP genotyping assays were used (probe ID: rs10818488, C_2783655_10). Amplification was performed in a 10 µl reaction volume containing 5 µl of TaqMan genotyping Master Mix and 0.125 µl of TaqMan genotyping assay mix using the Step One real-time PCR system (Applied Biosystems-Life Technologies, Carlsbad, California, USA). Thermal cycling conditions consisted of initial denaturation at 95 °C for 10minutes, followed by 40 cycles of 95 °C for 15 seconds and 60 °C for 1 minute.

Statistical Analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. Qualitative data were described using number and percent. Quantitative data were described using mean and standard deviation for normally distributed data while abnormally distributed data was expressed using median, minimum and maximum. Comparison between different groups regarding categorical variables was tested using Chi-square test. Odd ratio (OR) and 95% Confidence Interval were used. Significance of the obtained results was judged at the 5% level.[26]

Clinical and demographic data:

One hundred and seventy nine subjects; composed of 70 RA patients, 64 SLE patients and 45 healthy controls (mean age±SD= 39±11.20) years, participated in this study. Clinical and demographic data of patients are shown in Table (1).

IL-22 concentration in culture supernatants

There was a significant increase in concentration of IL-22 before and after treatment with PHA in RA patients when compared to control group. A significant difference between different activity groups was also seen. The same results were detected in SLE patients (Table 2).

Different correlation studies were performed between IL-22 before and after stimulation using PHA and various clinical and laboratory data. In RA patients; a significant positive correlation was found

between IL-22 levels and tender joints count and erythrocyte sedimentation rate (ESR). In addition a significant positive correlation was found between CRP and IL-22 without stimulation. However, In SLE patients; CRP only showed a positive correlation with IL-22 levels after stimulation with no other significant correlations with different parameters (Table 3).

Association of TRAF1 polymorphisms with autoantibodies and disease activities

After agreement to Hardy Weinberg test (Table 4) we analyzed TRAF1 polymorphism in both patients and controls statistically. In RA patients; the magnitude of association was increased in those patients who were autoantibody positive either RF+ or anti- CCP+. TRAF1 A allele was significantly associated with RF+ and anti-CCP+ RA patients when compared with control. (OR = 3.344 CI: 1.79-6.24, P <0.001, OR = 1.881 CI: 1.03-3.41 P <0.034) respectively (Tables 5).

In SLE patients, TRAF1 rs10818488 was not significantly different between patients and control (Table 6).

Comparison between TRAF1 genotyping, clinical and laboratory data

There was significant positive correlation between different TRAF1 genotypes, ESR, RF, disease activity score (DAS28), disease duration and tender joints count (Table 7).

In SLE group, different genotypes showed also positive correlations to some laboratory parameters (Table 8).

Table 1. Patients Demographic and Clinical Data.

	RA patients (n=70)	SLE (n=64)	Controls (n=45)
Demographic data:			
Age (years)	41.0 ± 11.63	30.50 ± 9.43	35.0 ± 10.28
Sex (male/female)	18/52	14/50	9/36
Disease duration (years)	3.0 (0.33 – 20.0)	4.85 (0.16-21)	
Clinical data			
ESR (1^{st}hr:) mm/hr	54.10 ± 29.82	65.21 ± 38.09	
CRP (mg/L)	15.0 (2.90 – 71.0)	11.85 (1.0 – 78.0)	
DAS28	4.58 ± 0.97		
Tender joints count (0-28)	8.0 (2.0 – 26.0)		
Swollen joints count (0-28)	2.0 (0.0 – 12.0)		
MHAQ	0.60 (0.12 – 2.25)		
RF (IU/mL)	15.0 (6.90 – 359.0)		
Anti-CCP (U/mL)	29.70 (12.10 – 178.0)		
ANA		160.0 (40.0 – 640.0)	
Anti-ds-DNA (IU/mL)		108.50 (10.10 – 857.0)	

MHAQ=Modified Health Assessment Questionnaire
Values was expressed as mean ± SD for parametric data or median (Min – Max.) for non-parametric data

Table 2. Concentration of IL-22 before and after treatment with PHA in RA and SLE patients with different disease activities and control group.

	Mild (n = 12)	Moderate (n = 34)	Severe (n = 24)	Control (n = 45)	RA patients (n=70)
IL-22 before stimulation with PHA	46.96±8.45	49.19±9.76	58.03±15.56	34.12±7.0	51.84±12.59
Sig. bet. grps	Mild – severe* (p = 0.015*), moderate – severe** (p = 0.009**), control –RA patients ** (p <0.001*)				
IL-22 after treatment with mitogen	61.06±12.94	66.79±15.82	79.02±20.03	51.83±14.43	70.0 ± 18.09
Sig. bet. grps	Mild – severe* (p = 0.010*), moderate – severe* (p = 0.025*), control –RA patients ** (p <0.001*)				
p	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**

Table 2. *Continued*

	Mild to moderate (n = 17)	Severe (n = 47)	Control (n = 45)	SLE patients (n= 64)
IL-22 before stimulation with PHA	30.01±5.84	28.59±6.71	34.12±7.0	28.97±6.48
Sig. bet. Grps	Moderate – control* (p= 0.034*), severe – control** (p<0.001**), control –SLE patients ** (p <0.001**)			
IL-22 after treatment with mitogen	48.03±4.95	46.73±5.64	51.83±14.43	47.08±5.45
Sig. bet. Grps	Severe – control* (p= 0.018*), control –SLE patients ** (p <0.001**)			
P	<0.001*	<0.001*	<0.001*	<0.001*

Values was expressed as mean ± SD for parametric data or median (Min – Max.) for non parametric data.
p: p value for comparing between before and after in each group.
*: Statistically significant at $p \leq 0.05$
**: Statistically significant at $p \leq 0.01$

Table 3. Correlation between IL-22 levels, clinical and laboratory data among RA and SLE patients.

Variable in RA patients	IL-22 without PHA		IL-22 with PHA	
	(r)	p	(r)	p
Tender joints count	0.366*	0.002*	0.322*	0.007*
Swollen joints count	-0.147	0.223	-0.218	0.070
ESR	0.332*	0.005*	0.323*	0.006*
CRP	0.326*	0.006*	0.208	0.084
RF	0.134	0.269	0.145	0.231
Anti-CCP	0.044	0.716	0.065	0.590
Variable in SLE patients	**IL-22 without PHA**		**IL-22 with PHA**	
	(r)	p	(r)	p
SLAEDI	-0.104	0.415	0.015	0.904
ESR	-0.103	0.417	-0.065	0.607
CRP	-0.042	0.740	-0.275*	0.028*
ANA	0.182	0.149	0.015	0.906
Anti DNA	-0.036	0.778	0.141	0.267

*: Statistical significance at p<0.05. Spearman coefficient test.

Table 4. The observed and expected values of the genotype frequencies among the studied groups.

Genotype	Observed	Expected	χ^2	P-value
RA cases				
G/G	18	21.7		
A/G	42	34.5	3.262	0.071
A/A	10	13.7		
SLE cases				
G/G	28	29.6		
A/G	31	27.9	0.809	0.368
A/A	5	6.6		
Control				
G/G	21	22.1		
A/G	21	18.9	0.556	0.456
A/A	3	4.1		

If P<0.05 - not consistent with HWE. Hardy Weinberg test

Table 5. TRAF1 polymorphismrs10818488 distributions in RA patients and control group.

Genotyping	Control	RA patients	Odds ratio (95%CI)	P-value	RF positive patients (>20IU/ml)	Odds ratio (95%CI)	P-value	Anti-CCP patients (>30IU/ml)	Odds ratio (95%CI)	P-value
A/A	3	10	3.889 (0.92-16.3)	0.054	6	-	<0.001*	4	3.889 (0.81-18.64)	0.076
A/G	21	42	2.333* (1.02-5.29)	0.041*	22	-	<0.001*	22	4.695* (1.47-14.93)	0.005*
G/G	21	18	-	0.021*	0	-	<0.001*	4	-	0.006*
wild type allele G	63	78	-	0.030*	22		<0.001*	30		0.034*
mutant allele A	27	62	1.855* (1.05-3.24)		34	3.344* (1.79-6.24)		30	1.881* (1.03-3.41)	

*: Statistical significance at p<0.05.

Table 6. TRAF1 polymorphismrs10818488 distributions in SLE patients and control group.

Genotyping	Control	SLE patients	Odds ratio (95%CI)	P-value	ANA positive patients	Odds ratio (95%CI)	P-value	Anti-DNA positive patients	Odds ratio (95%CI)	P-value
A/A	3	5	1.250 (0.26-5.82)	0.776	5	-	0.305	5	-	0.208
A/G	21	31	1.107 (0.50-2.4)	0.801	31	-	0.014*	21	0.700 (0.22-2.18)	0.539
G/G	21	28	-	-	23	-	-	21	-	-
wild type allele G	63	87		0.750	77		0.024*	63		0.702
mutant allele A	27	41	1.10 (0.61-1.97)		41	-		31	1.181 (0.503-2.77)	

Table 7. Comparison between TRAF1 genotyping, clinical and laboratory data.

	Genotype			P
	A/A	A/G	G/G	
ESR (1st hr:)	76.40±9.63	49.76±26.81	51.83±37.93	0.006*
CRP (mg/L)	14.8(14.8-71.0)	15.0(2.9-67.0)	21.0(3.0-48.0)	0.152
RF (IU/mL)	24.0(15.1-24.0)	24.0(6.9-359.0)	10.1(7.3-15.0)	<0.001*
Anti-CCP(U/mL)	29.7(29.7-30.1)	33.6(12.1-178.0)	21.9(18.0-175.4)	0.690
DAS28	5.43±0.74	4.48±0.91	4.34±0.99	0.001*
Tender joints count	4.0(4.0-26.0)	8.0(2.0-16.0)	6.0(2.0-14.0)	0.004*
Swollen joints count	2.0(2.0-2.0)	2.0(0.0-12.0)	2.0(0.0-3.0)	0.238
Disease duration (y)	1.0(1.0-4.0)	3.0(0.33-20.0)	2.0(1.0-6.0)	0.004*
MHAQ	0.60(0.50-2.25)	0.60(0.12-0.80)	0.60(0.13-1.50)	0.354

Values was expressed as mean±SD for parametric data or median (Min.-Max.) for non parametric data
*: Statistical significance at p<0.05
Normally quantitative data was expressed in (Mean±SD) and was compared using F test (ANOVA), while abnormally quantitative data expressed in Median (Min. – Max.) and was compared using Kruskal Wallis test

Table 8. Comparison between TRAF1 genotyping, clinical and laboratory data in SLE group.

	Genotype			P
	A/A	A/G	G/G	
ESR (1st hr:)	81.60±23.26	77.95±40.33	48.16±30.99	0.005*
CRP (mg/L)	2.97 (2.97 – 6.0)	24.0 (3.30 – 97.0)	11.70 (1.0 – 107.0)	0.004*
ANA (IU/mL)	80.0 (80.0 – 160.0)	160.0 (80.0 – 640.0)	80.0 (40.0 – 640.0)	0.037*
Anti DNA (U/mL)	95.0 (95.0 – 857.0)	122.0 (10.10 – 650.0)	142.50 (16.80 – 387.0)	0.456
SLEADI	15.0 (15.0 – 29.0)	15.0 (2.0 – 50.0)	24.0 (4.0 – 50.0)	0.829
Disease duration	3.0 (3.0 – 6.0)	3.0 (0.17 – 20.0)	3.0 (1.0 – 8.0)	0.802

Values was expressed as mean±SD for parametric data or median (Min – Max.) for non parametric data.
*: Statistically significant at p≤0.05

DISCUSSION

Cytokines play a fundamental role in the pathogenesis of RA and SLE. One of these cytokines is IL-22, a member of the IL-10 superfamily, is primarily produced by activated T cells. It is often secreted together with IL-17 by Th17. Upon binding to its receptor, IL-22 activates intracellular kinases (JAK1, Tyk2, and MAPK) and transcription factors (STAT3) that induce proliferative and anti-apoptotic pathways. Also it can initiate inflammatory immune responses and has been shown to induce production of CCL-2 and proliferation of synovial fibroblasts and osteoclasts in RA.[27]

This study clarified that IL-22 concentration in culture supernatants of lymphocytes before stimulation with PHA was significantly higher in RA and SLE patients than healthy controls.

Different studies found an increased in serum levels of IL-22 in RA patients.[28] On the other hand, decreased serum and plasma levels of IL-22 were observed in SLE patients in a previous study.[29] There are possible reasons for this discrepancy: different cell purification techniques and possibly as suggested by Ziesche et al.[30], glucocorticoid (dexamethasone) which is the treatment given to SLE patients in the previous study can suppress IL-22 production of plasma and PBMCs. Another study carried out by Qin et al.[31] showed that the percentages of IL-22 positive CD4+ T cells were increased in the PBMCs of patients with SLE compared with healthy control subjects and with a strong positive correlation to disease activity index (SLEDAI).

A highly significant increase in the concentration of IL-22 before stimulation with PHA was observed in patients with severe RA disease activity when compared separately to those of mild and moderate disease activity respectively. In accordance to our findings da Rocha et al.[28] reported that the levels of IL-22 correlated well with DAS28. Also Zhao et al.[32] in their study reported that the percentage of Th22 cells correlated positively with the levels of plasma IL-22 in RA patients and both of them correlated positively with DAS28.

Furthermore, the concentration of IL-22 after PHA stimulation was significantly higher in RA and SLE patients than healthy controls especially in severe disease activity groups.

Roeleveld DM et al. demonstrated that, not only the role of IL-22 in the initiation and augmentation of a spontaneous model of experimental arthritis by using gene knockout mice and neutralizing antibodies for IL-22 but also, the reduction of IL-17 serum levels after IL-22 blocking, suggesting that the observed feedback loop of IL-22 on Th17 cells is a target in RA treatment.[33]

Comparable results were obtained by Caiet al.[27] in patients with Behcet's disease, after stimulation with anti-CD3 and anti-CD28 antibodies, a considerably higher concentration of IL-22 was detected in PBMCs from BD patients than controls.

The importance of IL-22 has been highlighted also in the pathogenesis of psoriasis, IL-22 mediates keratinocyte activation via phosphorylation of STAT3, leading to acanthosis that is associated with a psoriatic phenotype.[34]

Hao et al.[35] showed that the concentration of IL-22 in serum and plasma from psoriatic patients was correlated with psoriasis area and severity index (PASI). Also Lavoie et al.[36] found that sera level of IL-22 was elevated in patients of Sjogren's syndrome in comparison to control group and also correlated with disease activity.

Whereas in IBD, IL-22 was found to be protective through maintaining the integrity of the epithelial barrier and protecting mucin-secreting goblet cells.[37]

Correlation analysis of our clinical data revealed a significant positive correlation between IL-22 levels

and ESR, CRP and tender joints count in RA patients and CRP only in SLE patients. This can be explained by the fact that ESR, CRP and tender joints count are used for assessment of disease activity in RA patients by DAS28 and that, IL-22 concentration was significantly changed with different disease activities. C-reactive protein is not only an inflammatory marker, but may also induce a proinflammatory effect by activating monocyte chemoattractant protein.[38] From these results laboratory and clinical data as ESR, CRP, tender joint counts and IL-22 can act as inflammatory biomarkers giving indication to the activity and degree of severity of RA.

TRAF1 gene encodes an important protein which acts as a mediator of the TNF and CD40 transduction pathways.[39] The presence of TRAF1 polymorphisms seems to affect the natural history of RA, increasing the risk of erosions. Our results clarified that both TRAF1 A/A and A/G genotypes displayed increased risk of RA witha significant increase in TRAF1 A allele in RA patients when compared to in control individuals and this result suggested an association of this polymorphism with the RA. These results agree with Zervou et al.[11], who suggested this region as RA associated region and Kurreeman et al.[40] in their study on Dutch population. On the contrary a genome-wide study performed by the Bruton et al.[41] failed to identify this region as a candidate for RA. Another study carried out by Ahmediou et al., in Iranian population suggested lack of association between TRAF1 and RA[39]. Therefore, rs10818488 TRAF1 might not be a genius allele related to RA in Asians and this can be explained by distinct population specific differences in the prevalence of this allele and the Iranian population is composed of different ethnic groups, so, identifying alleles that have lower frequency in this population is hardly possible.

On the other hand, we did not find a significant association between TRAF1 A allele and SLE and this was on the contrary of Zervou et al.[42] in his study on Turkish patients.

RA is a heterogeneous disease with a considerable variation in phenotype as evidenced by the fact that some patients are autoantibody-positive whereas others are not. These autoantibodies may be associated with genetic and environmental factors as indicators of disease outcome. The magnitude of association of TRAF1 polymorphism was increased in those patients who were autoantibody positive either RF+ or anti- CCP+. This agree with the results obtained by Kurreeman et al.[40] and Mohamed et al.[24], who found that the presence of this polymorphism in seropositive RA patients carrying the A allele is more than seronegative RA patients and normal subjects who carried the same allele.

Conclusion

IL-22 level is positively correlated with disease activity, tender and swollen joints count and ESR in RA and CRP in SLE. This indicates that IL-22 is associated with inflammatory process and it has a role in RA and SLE pathogenesis and prognosis.

Although there are inconsistent findings which need to be resolved, there are a synergism between TNF-α, IL-17 and IL-22, so, targeting IL-22 cytokine may be an effective therapeutic approach for chronic inflammation in the future.

Also TRAF1 was significantly associated with RF positive, anti-CCP positive patients, ESR and tender joints count and this confirm importance of TRAF1 in progression of RA.

REFERENCES

1. Yun Ye, Su-Liang Li, Ming Xie, Ping Jiang, Kai-Ge Liu, et al. Judging disease activity in rheumatoid arthritis by serum free kappa and lambda light chain levels. Kaohsiung J med sci 2013; 29 (10):547-53.
2. Gabriel SE, Michaud K. Epidemiological studies in incidence, prevalence, mortality, and comorbidity of the rheumatic diseases. Arthritis Res Ther. 2009; 11:229.
3. O'Neill S, Cervera R. Systemic lupus erythematosus. Best Pract Res ClinRheumatol. 2010; 24:841–55.
4. Bingham CO. The pathogenesis of rheumatoid arthritis: pivotal cytokines involved in bone degradation and inflammation. J Rheumatol 2002; 29: 3-9.
5. Mease P. TNF alpha therapy in psoriatic arthritis and psoriasis. Ann Rheum Dis 2004; 63:755-8.
6. Lee YH, Rho YH, Choi SJ, Ji J, Song GG. Association of TNF-alpha –308 G/A polymorphism with responsiveness to TNF--blockers in rheumatoid arthritis: a meta-analysis. RheumatolInt 2006; 27:157-61.
7. Kurreeman FA, Goulielmos GN, Alizadeh BZ, Rueda B, Houwing-Duistermaat J, et al. The TRAF1-C5 region on chromosome 9q33 is associated with multiple autoimmune diseases. Ann Rheum Dis. 2010; 69(4):696-9.
8. Finno CJ, Aleman M, Higgins RJ, Madigan JE, Bannasch DL. Risk of false positive genetic associations in complex traits with underlying population structure: a case study. Vet J. 2014; 202:543–9.
9. Belot A, Cochat P. Monogenic systemic lupus erythematosus. Nephrol Ther.2012; 8:1–4.
10. Gregersen PK, Olsson LM. Recent advances in the genetics of autoimmune disease. Annu Rev Immunol. 2009; 27:363-91.

11. Zervou MI, Sidiropoulos P, Petraki E, Vazgiourakis V, Krasoudaki E, et al. Association of a TRAF1 and a STAT4 gene polymorphism with increased risk for rheumatoid arthritis in a genetically homogeneous population. Hum Immunol. 2008; 69(9):567-71.
12. Ritchlin CT. Therapeutic considerations in spondyloarthritis patients who fail tumour necrosis factor antagonists. Best Pract Res ClinRheumatol 2010; 24:683-92.
13. Miossec P, Korn T, Kuchroo VK: Interleukin-17 and type 17 helper T cells. N Engl J Med 2009, 361:888 – 898 61.
14. O'Shea JJ, Kanno Y., Chan AC: In Search of Magic Bullets: The Golden Age of Immunotherapeutics . cell 2014, 227-40
15. Zenewicz LA, Flavell RA. Recent advances in IL-22 biology. IntImmunol 2011; 23:159-63.
16. Duhen, T, Geiger R, Jarrossay D, Lanzavecchia A, Sallusto F. Production of interleukin 22 but not interleukin 17 by a subset of human skin-homing memory T cells. Nat Immunol 2009; 10: 857–63.
17. Kagami S, Rizzo HL, Lee JJ, Koguchi Y, Blauvelt A. Circulating Th17, Th22, and Th1 cells are increased in psoriasis. J Invest Dermatol 2010; 130: 1373-83.
18. Eyerich S, Wagener J, Wenzel V, ScarponiC,Pennino D, et al. IL-22 and TNF-α represent a key cytokine combination for epidermal integrity during infection with Candida albicans. Eur J Immunol 2011; 41: 1894-901.
19. Classen A, Kalali BN, Schnopp C, Andres C, Aguilar-Pimentel JA, et al. TNF receptor I on human keratinocytes is a binding partner for staphylococcal protein A resulting in the activation of NF kappa B, AP-1, and downstream gene transcription. Exp Dermatol 2011; 20: 48-52.
20. Neogi T, Aletaha D, Silman AJ, Funovits, Felson DT, Bingham CO, et al. The 2010 American College of Rheumatology/European League Against Rheumatism classification criteria for rheumatoid arthritis. Arthritis Rheum 2010; 62(9):2582-91.
21. Petri M, Orbai AM, Alarcón GS, Gordon C, Merrill JT, et al. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum. 2012; 64: 2677-86.
22. Van der Heijde DM, van 't Hof MA, van Riel PL, Theunisse LA, Lubberts EW, van Leeuwen MA, et al. Judging disease activity in clinical practice in rheumatoid arthritis: first step in the development of a disease activity score. Ann Rheum Dis. 1990;49:916-20.
23. Griffiths B, Mosca M, Gordon C. Assessment of patients with systemic lupus erythematosus and the use of lupus disease activity indices. Best Pract Res Clin Rheumatol. 2005; 19:685-708.
24. Mohamed RH, Pasha HF, El-Shahawy EE. Influence of TRAF1/C5 and STAT4 genes polymorphisms on susceptibility and severity of rheumatoid arthritis in Egyptian population. Cell Immunol 2012; 273:67-72.
25. Perper RJ, Zee TW, Mickelson MM. Purification of lymphocytes and platelets by gradient centrifugation. J Lab Clin Med 1986; 72:842-8.
26. Kirkpatrick LA, Feeney BC. A simple guide to IBM SPSS statistics for version 20.0. Student ed. Belmont, Calif.: Wadsworth, Cengage Learning; 2013.
27. Cai T, Wang Q, Zhou Q, Wang C, Hou S, et al. Increased Expression of IL-22 is associated with disease activity in Behcet's Disease. PLoS One 2013; 8(3):e59009.
28. Da Rocha Jr LF, Duarte AL, Dantas AT, Mariz HA, Pitta ID, et al. Increased serum interleukin 22 in patients with rheumatoid arthritis and correlation with disease activity. J Rheumatol 2012; 39(7):1320-5.
29. Cheng F, Guo Z, Xu H, Yan D, Li Q. Decreased plasma IL22 levels, but not increased IL17 and IL23 levels, correlate with disease activity in patients with systemic lupus erythematosus. Ann Rheum Dis 2009; 68:604–6.
30. Ziesche E, Scheiermann P, Bachmann M, Sadik CD, Hofstetter C, et al. Dexamethasone suppresses interleukin-22 associated with bacterial infection in vitro and in vivo. Clin Exp Immunol 2009; 157:370–6.
31. Qin WZ, Chen LL, Pan HF, Leng RX, Zhai ZM, et al. Expressions of IL- 22 in circulating CD4+/CD8+ T cells and their correlation with disease activity in SLE patients. ClinExp Med 2011; 11: 245–50.
32. Zhao L, Jiang Z, Jiang Y, Ma N, Zhang Y, et al. IL-22+ CD4+ T cells in patients with rheumatoid arthritis. Int J Rheum Dis 2013; 16 (5):518-26
33. RoeleveldDM ,Marijnissen RJ , Van den Berg WB, Koenders MI . IL-22 drives the initiation and augmentation of TH 17 - dependent experimental arthritis . Ann Rheum Dis 2014;73
34. Zheng Y, Danilenko DM , Valdez P et al., "Interleukin-22, a TH17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis," Nature 2007, 445; 7128:648–51
35. Hao JQ .Targeting IL22 in psoriasis. Inflammation 2014;37(1):94-9.
36. Lavoie TN, Stewart CM, Berg KM, Li Y, Nguyen CQ. Expression of interleukin-22 in Sjögren's syndrome: significant correlation with disease parameters. Scand J Immunol 2011; 74(4):377-82.
37. Zenewicz LA, Yancopoulos GD, Valenzuela DM, Murphy AJ, Stevens S, Flavell RA: Innate and adaptive interleukin-22 protects mice from inflammatory bowel disease. Immunity 2008, 29:947–95

38. Yeh ET: CRP as a mediator of disease. Circulation 2004; 109 (21 Suppl 1):11-4.
39. Ahmadiou S, Akhiani M, Salimzadeh A, Keramatipour M. Lack of Association between Single Nucleotide Polymorphism rs10818488 in TRAF1/C5 and rheumatoid arthritis in Iranian Population. Iran J Asthma Allergy Immunol 2014; 13(1):9-25.
40. Kurreeman FA, Padyukov L, Marques RB, Schrodi SJ, Seddighzadeh M, Stoeken-Rijsbergen G, et al. A candidate gene approach identifies the TRAF1/C5 region as a risk factor for rheumatoid arthritis PLoS Med 2007; 4 :1515–24.
41. Burton PR, Clayton DG, Cardon LR, Craddock N, Delovkas P, et al. The Wellcome Trust Case Control Consortium, Genome-wide association study of 14,000 cases of seven common diseases and 3000 shared controls, Nature 2007;447: 661–78.
42. Zervou MI, Vazgiourakis VM, Yilmaz N, Kontaki E, Trouw LA, et al. TRAF1/C5, eNOS, C1q, but not STAT4 and PTPN22 gene polymorphisms are associated with genetic susceptibility to systemic lupus erythematosus in Turkey. Hum Immunol 2011; 72(12):1210-3.