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ORIGINAL ARTICLE

The role of interleukins 4, 17 and interferon gamma as biomarkers in patients with Systemic Lupus Erythematosus and their correlation with disease activity

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KEYWORDS

Systemic Lupus Erythematosus;
Cytokines;
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Abstract *Aim of the work:* This work was designed to study the production of proinflammatory cytokines in SLE patients and their correlation with disease activity and study if they can be used as biomarkers for renal activity in lupus nephritis patients.

Patients and methods: This study was carried out on 70 subjects divided into two groups: Group I (SLE group) which included 40 SLE patients and Group II (Control group) which included 30 apparently healthy controls. The patients were subjected to full history taking and complete clinical examination. Assessment of disease activity in SLE patients by Systemic Lupus Erythematosus Disease Activity Index (SLEDAI). Sera of patients and controls were screened for the level of cytokine expression of T helper cells including interleukin 17 (IL-17), interleukin 4 (IL-4) and interferon gamma (IFN-γ).

Results: Serum levels of IL-4 were significantly lower while both IL-17 and IFN-γ were significantly higher in SLE patients than in the control group. The most powerful predictor and correlated cytokine with the SLEDAI in SLE patients was IL-17. Higher serum level of IFN-γ was associated with more pyuria and hematuria, while higher IL-17 was associated with more pyuria and proteinuria in SLE patients.

Conclusion: The serum level of IL-17 and IFN-γ was proven to be significantly higher in SLE patients and can be used as biomarkers of renal activity.

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1. Introduction

Systemic Lupus Erythematosus (SLE) is an autoimmune disease associated with chronic immune activation and tissue damage that results from the deposition of immune complexes and infiltration of activated T cells into susceptible organs [1].

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Cytokines are soluble factors which play a role in differentiation, maturation, and activation of various immune cells. They are not only involved in immune dysregulation of SLE, but also in local inflammatory response leading to tissue injury [2]. These cytokines may exert either pro or anti-inflammatory effects, or both, depending on specific local microenvironment, thus contributing greatly to SLE pathogenesis. Understanding these cytokine abnormalities may be beneficial in developing effective targeting therapeutics [3]. Cytokines play an important role in lupus nephritis, so use of cytokines as biomarkers of disease activity in SLE and lupus nephritis is of particular interest [2]. T cells can be subdivided by the patterns of cytokine released into: (1) Th1 cells produce IL-2 and IFN-γ, which are critical for cell-mediated immunity. (2) Th2 cells produce IL-4, IL-5 and IL-10, which promote antibody production and humoral immunity. (3) Th17 cells produce IL-17 [4].

IFN-γ is generated by both innate and acquired immune cells, particularly T cells and natural killer (NK) cells. It is commonly accepted that IFN-γ can promote Th1 polarization, facilitate specific cytotoxicity by increasing the expression of MHC class-I and -II molecules, and boost antigen processing and immunoglobulin switching [5]. Targeting therapy for IFN-γ has been successfully applied to lupus mice, and treatment with humanized anti-IFN-γ mAb or recombinant IFN-γ-Ig fusion protein may provide a novel therapeutic strategy for this intractable disease in human [6].

IL-4 is a 17 kDa monomeric glycoprotein of the type hematopoietin superfamily secreted by Th2 cells, NK T cells, mast cells and basophils [7]. It plays a central role in regulating the differentiation of antigen-stimulated naive T cells to develop into IL-4-producing Th2 through IL-4R-mediated signaling [8]. IL-4 is a multifunctional cytokine. Although most studies have focused on the B-cell stimulatory and Th2 promoting properties of IL-4 in the development of autoantibodies and autoantibody-mediated diseases, a few reports suggest a T-cell suppressor role for this cytokine in lupus. These properties of IL-4 may sometimes result in opposing outcomes, amplifying or inhibitory, on overall B-cell functions [9].

IL-17 is a type I transmembrane protein isolated initially from a rodent CD4+ T cell DNA library [10]. It is mainly produced by activated Th17 cells which are in fact a subset of CD4+ T lymphocytes named after its hallmark cytokine IL-17. Recent data indicate that IL-17 driven inflammation amplifies SLE-induced tissue damage and contributes to tolerance breakdown in SLE patients [11]. Elevated IL-17 levels in SLE probably contribute to the recruitment and activation of immune cells (e.g., neutrophils and T cells) to target organs and thus amplify immune response [12]. The purpose of this work was to study the production of the cytokines in SLE patients and their correlation with disease activity and study if these cytokines can be used as biomarkers for renal activity in lupus nephritis patients.

2. Patients and methods

This study was carried out in Rheumatology and Rehabilitation Department, Faculty of Medicine, Zagazig University on 70 subjects divided into two groups: Group I (SLE group) which included 40 SLE patients diagnosed according to the American College of Rheumatology (ACR) classification criteria for SLE [13]. Group II (Control group) included 30 apparently healthy controls. Clinical examination as well as routine laboratory investigations confirmed their healthy state. Written informed consent was obtained from all patients and controls for their study participation. The study was approved by the local ethics committee of Zagazig University Hospitals.

2.1. Clinical examination

Patients were subjected to full history taking and complete clinical examination including general, locomotor system, skin, cardiovascular, chest, neurological and vascular examinations.

2.2. Disease activity

The disease activity was assessed in SLE patients by Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) [14]. Activity categories were defined on the basis of SLEDAI scores [15]; No activity (SLEDAI; 0), Mild activity (SLEDAI; 1–5), Moderate activity (SLEDAI; 6–10), High activity (SLEDAI; 11–19), and Very high activity (SLEDAI; 20).

2.3. Investigations

Investigations included are complete blood picture, erythrocyte sedimentation rate, C-reactive protein, complete urine analysis, 24 h proteinuria, liver and kidney function tests, Complement 3 (Normal value: 87–187 mg/dl), Complement 4 (Normal value: 16–38 mg/dl), ANA, and Anti DNA double stranded antibody (Positive value up to 25 IU/ml).

2.4. Cytokines assay

Blood samples from patients and controls were centrifuged and the sera screened for the level of cytokine expression of T helper cells including interleukin 17, interleukin 4 and interferon gamma (IFN-γ). Sera were analyzed for cytokines by sandwich enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's protocols. IL-4 assay by Ani Biotech Oy, Orgenium Laboratories Business Unit, Finland, Product code IL10001, IL04001 and IL13001. Assay range: 15.6–250 pg/ml. IFN-γ assay by Invitrogen, USA, Catalog No. KAC1231. Assay range: 0–1.2 IU/ml. IL-17 assay by WKEA MED supplies, USA. Assay range: 0.5–15 ng/L.

Statistical analysis: It was performed using SPSS statistical software, version 11.0 (SPSS, Chicago, IL). Quantitative variables were given as means ± SD, medians, range and categorical variables in frequencies and percents. *t* Test or the Wilcoxon rank-sum tests were used for continuous variables according to the distribution of the variable and the χ^2-test for categorical variables. Measures the closeness of the association between two quantitative continuous variables by correlation coefficient. Kruskall Wallis one way analysis of variance (KW) test was used to compare median for >2 independent samples that are not related. *P* value less than 0.05 was considered statistically significant.

3. Results

3.1. Demographic data of patients and control groups

Demographic data of patients and control groups are presented in Table 1.

3.2. Clinical manifestations of SLE

It was observed that the most frequent clinical variables among SLE patients at the time taking samples were malar rash, arthritis then fever representing (77.5%), (60%) and (55%), respectively and the least frequent clinical variables are cranial nerve affection and organic brain syndrome forming 0% for both. Visual changes were observed in 2.5% in the form of retinal changes, cytoid bodies, retinal hemorrhages, serous exudate or hemorrhage in choroid, optic neuritis (not due to hypertension, drugs, or infection) (Fig. 1).

3.3. Laboratory investigations among SLE patients

Laboratory investigations among SLE patients are presented in Fig. 2.

3.4. Disease activity of SLE patients

The median SLEDAI of our SLE patients was 8 with a range from 0 to 30. Percentage of activity categories is represented in graph, while inactive category was 5%. The majority of our patients had moderate activity of the disease (Fig. 3).

3.5. Serum levels of cytokines in SLE and control group

Comparison between the serum levels of IL-4, IL-17 and IFN-γ in SLE and control group revealed that serum levels of IL-4 were significantly lower in SLE patients than in normal subjects. The serum levels of both IL-17 and IFN-γ in SLE patients were significantly higher than the levels in the control group Table 2.

3.6. Sensitivity and specificity of cytokines in SLE patients

IL-4 was found to be the most sensitive cytokine among SLE patients (Table 3).

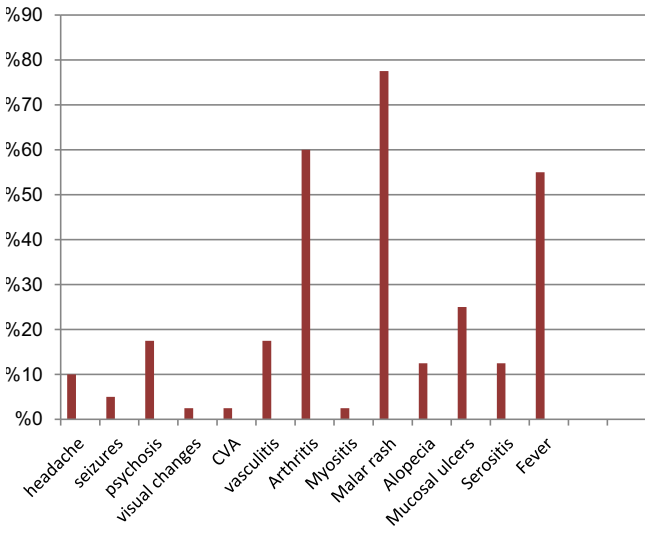
3.7. Association between serum cytokine levels and clinical presentations in SLE patients

There was an association between elevated IL-17 levels and vasculitis (in the form of ulceration, gangrene, tender finger nodules, periungual infarction, splinter hemorrhages), mucocutaneous manifestations and serositis. Elevated serum IFN-γ was found to be associated with arthritis and mucocutaneous manifestations. IL-4 was found to have no association with any of the clinical presentations of SLE (not shown).

Table 1 Demographic data of patients and control groups.

Item	Patients (n = 40)	Control (n = 30)	T	p
Age (years)				
Range	17–50	20–53	t = 1.04	0.68 (NS)
Mean ± SD	31 ± 9.95	27 ± 10.4		
Sex F/M	38/2	29/1	0.12 (X^2)	0.74 (NS)
Disease duration (years)				
Range	1–20	–		
Median	3	–		

$p > 0.05$ = Non significant (NS), $p < 0.05$ = significant.



CVA; cardio-vascular accident

Figure 1 Clinical manifestations of the SLE patients.

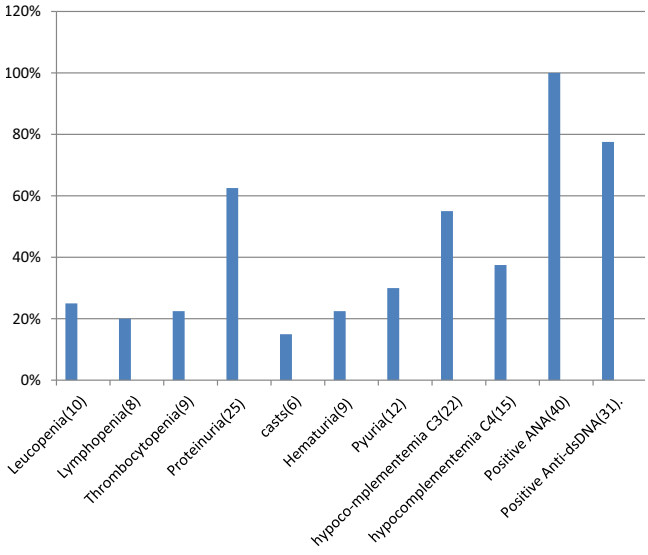


Figure 2 Laboratory investigations among the SLE patients.

3.8. Correlation of serum cytokines levels with anti-dsDNA, C3, C4 and SLEDAI

There was no correlation of IL-4 serum level with SLEDAI, or any of the laboratory parameters in SLE group, while there was a significant correlation between serum levels of both IL-17 and IFN-γ as well as the SLEDAI and anti-dsDNA. There was a negative significant correlation between C4 and both IL-17 and IFN-γ. Correlation of the levels of the three cytokines with C3 showed that only IL-17 was negatively correlated (Table 4).

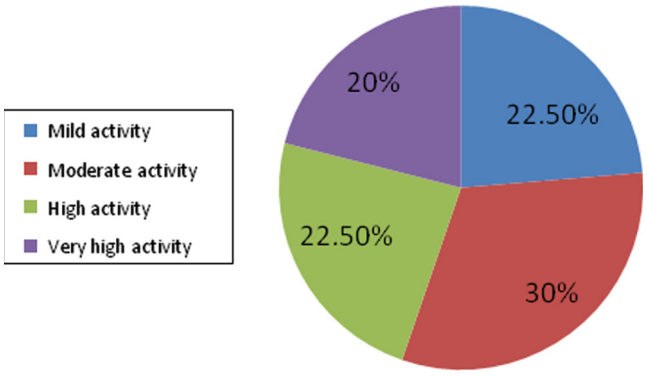


Figure 3 SLE Disease Activity Index (SLEDAI) subgroups of SLE patients.

Table 2 Serum levels of cytokines IL-4, IL-17 and IFN-γ in SLE and control group.

Cytokine	Patients (n = 40)	Control (n = 30)	P
IL-4 (pg/ml)	1.2 ± 0.6 (0.1–4.3)	4.4 ± 0.83 (3.1–5.9)	0.00*
IL-17 (ng/L)	37 (0.5–102)	6.75 (0.5–14)	0.00**
IFN-γ (IU/ml)	0.7 (0.2–1.7)	0.29 (0.2–0.37)	0.00**

IL: interleukin, IFN-γ: interferon gamma.
* t-test.
** Mann–whitney test.

3.9. Cytokines levels among SLEDAI subgroups

From Table 5 serum concentrations of IL-4 were not significantly different among the five subgroups of SLEDAI (p = 0.33), while there were significant differences in serum concentrations of both IL-17 and IFN-γ between mild activity and very high activity groups with serum concentration of both cytokines being significantly higher in very high activity group. By entering the previous correlated factors with SLEDAI in the multiple forward stepwise logistic regression analysis, we found that IL-17 is the most powerful predictor and correlated cytokine with the SLEDAI in SLE patients.

3.10. Association between serum cytokine levels and renal activity descriptors of the SLEDAI

It was revealed that higher serum level of IFN-γ was associated with more pyuria and hematuria, while higher IL-17 was associated with more pyuria and proteinuria in the SLE patients (Table 6).

4. Discussion

In our work, we revealed that IL-4 is the most sensitive cytokine in SLE patients and by comparing between its serum levels in SLE and control group, they were significantly lower in SLE patients. This was in agreement with other studies [16–18] who found a suppressed expression of Th2 cytokine IL-4 in SLE patients. Also, Csiszâr et al. [19] found that the number of mRNA transcripts of IL-4 decreased significantly in the SLE group. On the other hand, there was an increased serum level of IL-4 in SLE patients in other studies [20,21]. While, similar levels of IL-4 in SLE patients compared with controls were observed by others [22,23]. IL-4 promotes antibody production by B cells, so it is expected to have higher levels in SLE patients. But its levels may not increase, as in our study, and this may be due to that IL-4 provides B cell help for non-complement-fixing antibodies while in SLE autoantibodies are of complement-fixing type [19]. In addition, there is Th1 predominence in SLE patients and this Th1/Th2 imbalance may limit the secretion of IL-4 levels [18]. There were elevated serum IL-17 levels among SLE group more than in the control group and this finding was in agreement with other different studies [14,21,24,25].

Serum levels of IFN-γ in SLE group were significantly higher than in normal subjects. Other studies go ahead with our results [19,20,22]. In contrast to our study, Yu and Wang [26] reported that the levels of IFN-γ were lower in SLE group than in control group. They explained their result by that Th1 or Th2 cells may have different functions in different phases of the disease. Besides, it can be explained by the small number of patients in their study which was only 20 and the short disease duration ranging between 1 and 35 months.

In correlation of serum cytokine levels with anti-dsDNA, we found that there was a significant positive correlation with IL-17 and this was in agreement with Dong et al. [27] who reported that the increase in anti-dsDNA induced by IL-17 was dose-dependent and could be completely blocked by IL-17 monoclonal antibody, but it cannot induce the peripheral blood mononuclear cell (PBMC) of normal controls to augment anti-dsDNA secretion. This suggested that the IL-17 effect on increased anti-dsDNA level by PBMC may be dependent on the characteristics of immunological and genetic abnormalities occurring in PBMC of lupus nephritis patients. IFN-γ had a significant positive correlation with anti-dsDNA titer which was supported by other studies [7,28]. On the other hand, no correlation between IL-4 and anti-dsDNA in SLE patients was in agreement with Csiszâr et al. [19].

Serum concentrations of IL-4 were not significantly different among the five subgroups of SLEDAI and there was no correlation between IL-4 and SLEDAI. Our results were consistent with other studies [19,22,29] and inconsistent with the

Table 3 Diagnostic properties of IL17, IL-4, and IFN-γ assays using optimal cutoff values.

Assay (cut off)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC	95% CI	P
IL-17 (⩾11.5)	77.5	83.3	86.1	73.5	0.811	0.701–0.922	<0.05
IL-4 (⩽2.5)	100	100	100	100	0.25	0.000–0.073	<0.05
IFN-γ (⩾0.38)	97.5	100	100	96.7	0.975	0.927–1.000	<0.05

IL: interleukin, IFN-γ: interferon gamma, PPV: positive predictive value, NPV: negative predictive value, AUC: area under the ROC curve, CI: confidence interval.

Table 4 The correlation between serum cytokine levels and anti-dsDNA, C3, C4 and SLEDAI.

Item	IL-4		IL-17		IFN-γ	
	r	*p*	*r*	*p*	*r*	*p*
Anti-dsDNA	0.26	0.10	0.59	0.000*	0.55	0.03*
C3	0.26	0.1	−3.34	0.03*	0.00	0.98
C4	−1.4	0.39	−0.65	0.04*	−0.49	0.02*
SLEDAI	0.10	0.52	0.85	0.01*	0.51	0.02*

IL: interleukin, IFN-γ: interferon gamma, C: complement, SLEDAI: SLE Disease Activity Index.
* Significant.

study of Yu and Wang [26] who found slightly lower levels in an inactive group. Our different results may be due to that their study was on IL-4 production by NK T cells of SLE patients and not serum level. There was a significant positive correlation between serum levels of IFN-γ and SLEDAI and there were significant differences in serum concentrations of IFN-γ between mild and very high activity groups being significantly higher in the very high activity group. This was in agreement with Gigante et al. [30], Csiszâr et al. [19], and Viallard et al. [29]. On the other hand, El-Sayed et al. [22] and Harigai et al. [31] found no correlation between IFN-γ and SLEDAI. There was a significant correlation between IL-17 serum levels and SLEDAI. This was consistent with Chen et al. [24] and Doreau et al. [12], while, Zhao et al. [25] found no significant difference.

In our study, we found that the most powerful predictor and correlated cytokine with the SLEDAI in SLE patients was IL-17. This result was supported by many other studies [12,21,24,25,32] who reported that IL-17 production is increased in patients with SLE. Also, Nalbandian et al. [33] explained increased secretion of IL-17 as a consequence of systemic inflammation and facilitated pathways that guide T cell differentiation into IL-17-producing cells (either Th17 or DN T cells) in SLE patients.

In our work, we found that IL-17 was associated with proteinuria and pyuria in SLE patients. Our results were supported by the work of Crispin et al. [34] who reported that IL-17 could be detected in significant amounts in analyzed renal biopsies from patients with lupus nephritis. On the other

Table 5 Comparison between serum cytokine levels among SLEDAI subgroups.

Cytokine	SLEDAI					*K***	*p*
	Inactive *n* = 2	Mild *n* = 9	Moderate *n* = 12	High *n* = 9	Very high *n* = 8		
IL-17							
Median (range)	7.75 (0.5–15)	20 (0.5–29)	30 (0.5–48)	60 (50–80)	87.5 (1.5–10)	26.1	0.00*
IL-4							
Median (range)	0.25 (0.1–0.4)	0.99 (0.4–1.9)	1.0 (0.2–1.7)	0.90 (0.3–4.3)	1.05 (0.3–1.7)	4.58	0.33
IFN-γ							
Median (range)	0.4 (0.4–0.4)	0.4 (0.07–1.1)	0.7 (0.2–1)	0.91 (0.15–4.3)	1.45 (0.7–1.6)	22.2	0.00*

IL: interleukin, IFN-γ: interferon gamma, SLEDAI: SLE Disease Activity Index.
* Significant.
** Kruskall Wallis one way analysis of variance (KW) test.

Table 6 Association between serum cytokine levels and renal activity descriptors of the SLEDAI in the SLE patients.

		IL-4	IL-17	IFN-γ
Sterile pyuria•	Absent (*n* = 28)	0.9 (0.1–4.3)	29.5 (0.5–96)	0.6 (0.07–4.3)
	Present (*n* = 12)	0.95 (0.3–1.8)	62.5 (15–102)	0.95 (0.15–1.6)
	*p***	0.96	0.03*	0.02*
Cast	Absent (*n* = 34)	0.9 (0.1–4.3)	32.5 (0.5–96)	0.7 (0.07–4.3)
	Present (*n* = 6)	0.83 (0.3–1.8)	57.5 (1.5–102)	0.85 (0.3–1.6)
	*p***	0.97	0.35	0.51
Hematuria	Absent (*n* = 31)	0.9 (0.1–4.3)	30 (0.5–102)	0.7 (0.07–4.3)
	Present (*n* = 9)	1 (0.3–1.8)	65 (1.5–100)	1.1 (0.15–1.5)
	*p***	0.92	0.75	0.04*
Proteinuria (> 0.5 g/24 h urine)	Absent (*n* = 15)	0.85 (0.1–1.5)	21.5 (0.5–83)	0.6 (0.4–1.62)
	Present (*n* = 25)	1 (0.2–4.3)	45 (7.5–102)	0.85 (0.07–4.3)
	*p***	0.32	0.02*	0.07

IL: interleukin, IFN-γ: interferon gamma.
* $p < 0.05$, significant.
** Mann–whitney test.
• Urinary tract infections were excluded.

hand, Zhao et al. [25] disagreed and found no significant difference in serum IL-17 level between SLE patients with and without nephritis. The higher serum level of IFN-γ was associated with pyuria and hematuria.

This result was supported by the work of Peterson et al. [35] who reported that the interferon gene signature had been found in glomerular tissue, suggesting local organ involvement of interferon γ. Also, Gigante et al. [30] reported that urinary IFN-γ levels were correlated with disease activity. So, both IFN-γ and IL-17 were found to be associated with the SLEDAI descriptors of renal activity indicating that they play a role in lupus nephritis.

In conclusion, the serum level of IL-17 and IFN-γ was proven to be significantly higher in SLE patients and can be used as biomarkers of renal activity.

Conflict of interest

There is no conflict of interest of the authors.

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