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Original Article

Elevated plasma levels of IL-12 and IFN-γ in systemic lupus erythematosus

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Abstract: Interleukin (IL)-12 and interferon (IFN)-γ are involved in the pathogenesis of a number of autoimmune and inflammatory diseases. In this study, we analyzed plasma expression of these two cytokines in systemic lupus erythematosus (SLE) patients, as well as their potential as biomarkers for SLE. In this study, we detected the plasma IL-12 and IFN-γ levels by enzyme linked immunosorbent assay (ELISA) in 44 patients with SLE and 44 healthy controls, results from our studies showed that the elevated plasma expression levels of IL-12 and IFN-γ were observed in SLE patients compared with healthy subjects (P<0.001 and P=0.013, respectively). Significantly positive correlation was found between IL-12 and IFN-γ (r=0.588, P<0.001). The area under curve (AUC) for IL-12 and IFN-γ were (0.756, 95% CI: 0.654-0.858, P<0.001) and (0.653, 95% CI: 0.538-0.769, P=0.013), respectively. To conclude, elvated plasma levels of IL-12 and IFN-γ in SLE suggest their potential role in this disease. In addition, plasma IL-12 and IFN-γ may serve as auxiliary indexes for SLE diagnosis.

Keywords: Systemic lupus erythematosus, cytokines, IL-12, IFN-γ, autoantibodies

Introduction

Systemic lupus erythematosus (SLE) is a prototypic autoimmune disorder, characterized by the production of multiple polyclonal autoantibodies and the presence of multiple cellular and molecular abnormalities in the immune system, which usually leads to intense inflammation and multisystemic tissue damage [1, 2].

Although the exact etiology of SLE is still unknown, the defect of some Th1 derived cytokines which mainly activate the cellular machinery of the immune system, is deemed to contribute directly to the pathogenesis of the disease [3-7]. Interleukin (IL)-12 and interferon (IFN)-γ were the most important cytokines in Th1 derived cytokines family. The main cellular targets of IL-12 are T cells and NK cells [8], it plays a key role in the differentiation of Th1 cells, which can specifically stimulate the signal transducer and activator of transcription 4 (STAT4) and transcription factor, thereby promote the differentiation of naive T cells toward the Th1 phenotype whatever in vivo or in vitro [9, 10], additionally, it promotes the secretion of IFN-γ, which as major cytokine secreted by Th1 cells, and IFN-γ can activate the macrophages to produce NO synthase and subsequently release reactive nitrogen species that promote the phagocytic function and local inflammation [11]. Except the biological oxidation functions, IFN-γ can also promote the secretion of IL-12 [12, 13], and induce the production of autoantibodies and soluble B lymphocyte stimulating factor, which promotes the activation of B cells [14]. Although increased levels of IFN-γ and IL-12 were found in SLE patients compared with controls [6, 15-17], there are also studies showing that IFN-γ and IL-12 were not increased [18-20]. So the expression levels of IL-12 and IFN-γ in SLE is worthy of being further studied and discussed. A predominance of these two Th1 cytokines is likely to lead to the inflammatory process of SLE [21].

Therefore, the plasma levels of IL-12 and IFN-γ were detected in SLE patients compared with

Table 1. Characters of patients and controls

Variable	SLE patients (n=44)	Healthy controls (n=44)	*P*
Age (years, mean ± SD)	34.91 ± 10.00	37.18 ± 11.18	NS
Male/female	2/42	2/42	NS
Anti-dsDNA positive, N (%)	16 (36.36)	NA	NA
Renal disease (with/without)	15/29	NA	NA

SLE, systemic lupus erythematosus; SD, standard deviation; NS, Non-significant; NA, not available.

healthy controls in the present study. Furthermore, the potential of these two cytokines as biomarkers for SLE were evaluated with the receiver operating characteristic (ROC) curve analysis.

Patients and methods

Patients and controls

Forty four patients (42 females and 2 males) fulfilling the revised 1997 American College of Rheumatology classification criteria for SLE [22] were recruited from the Department of Rheumatology at the First Affiliated Hospital of Anhui Medical University and Anhui Provincial Hospital. The female to male ratio was 42:2. The mean (SD) age was 34.91 ± 10.00 years.

For each patient with SLE, one control was randomly selected from among healthy people (without history of autoimmune disorders, major infection, and other inflammatory diseases), matched for sex, ethnic group, and sample availability. Healthy controls (HC; N=44) were recruited from healthy volunteer. The mean (SD) age was 37.18 ± 11.18 years (**Table 1**).

The basic demographic data (sex, ethnic group and age, etc), clinical data, and laboratory data were obtained by reviewing medical records or a questionnaire and reviewed by experienced physicians.

Each participant provided written informed consent which was approved by Ethics committee of Anhui Medical University.

IL-12 and IFN-γ system determination

Plasma was obtained from 2 ml of whole blood during the first visit of the patient in 4 hours. Plasma levels of IL-12 and IFN-γ were determined by an enzyme linked immunosorbent assay (ELISA) with commercial reagent kits (Shanghai yuan ye Biological Technology Co., Ltd. Shanghai, China). The assays were performed according to the manufacturer's protocols. Briefly, serum or cytokine standards were added to 96 well plate followed by an hour incubation at 37°C with HRP Conjugated reagent. The plate was washed and incubated with their corresponding biotinylated anticytokine detecting antibody for 30 min. They were developed by adding avidin peroxidase and peroxidase substrate. Absorbance was measured using ELISA reader at 450 nm, a standard curve for each assay was generated and cytokine production was calculated. The assays were performed in duplicate, and the means were used in further analysis.

Statistical analysis

Patient characteristics were expressed using frequency or descriptive analysis. The plasma IL-12 and IFN-γ levels between patients and control were compared using independent Student's t test. The correlations of IL-12 with IFN-γ were calculated using Pearson's correlation test in SLE patients and healthy controls. Finally, the receiver operating characteristic (ROC) curve analysis was used to analyze the prediction on SLE and the best cut off values of IL-12 and IFN-γ. Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) were computed for each cut off point of IL-12 and IFN-γ. The *P* value <0.05 was deemed as significant. The statistical analysis was presented using the IBM SPSS Statistics, Version 22.0 (IBM Company, Armonk, New York, USA). ROC was performed with medCalc.

Results

IL-12 and IFN-γ plasma level prevalence

As shown in **Figure 1**, the IL-12 plasma level in SLE patients was significant higher than in the normal controls (19.6464 ± 5.6560 versus 13.2792 ± 6.2767 (pg/ml), P<0.001). The IFN-γ plasma level in SLE patients was also significant higher than in the normal controls (293.7754 ± 113.7009 versus 241.9844 ± 81.4744 (pg/ml), P=0.013).

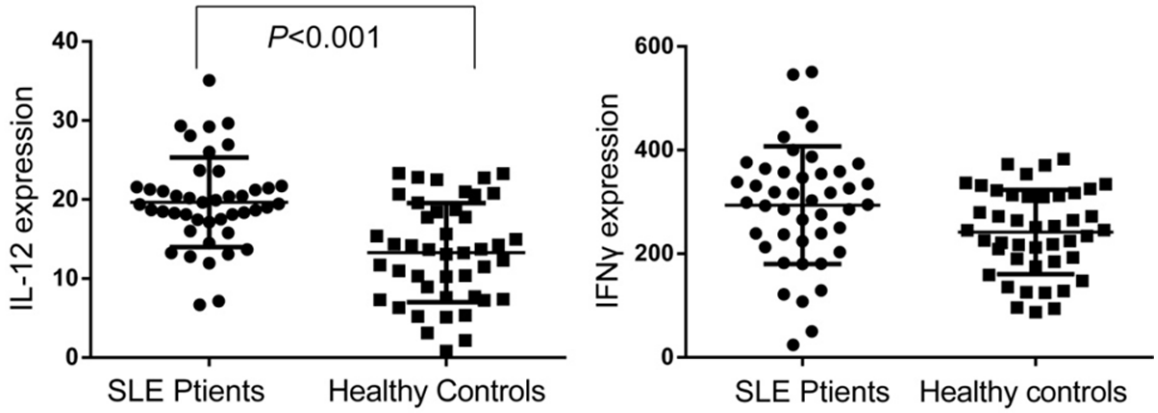

Figure 1. Levels of cytokines in patients with SLE and in healthy controls.

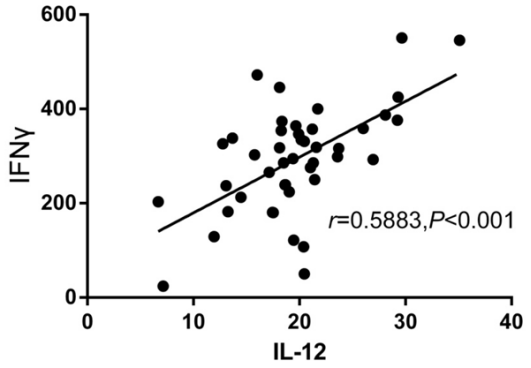

Figure 2. The correlation between the IL-12 levels with IFN-γ expression in SLE patients.

Moreover, IL-12 levels correlated with IFN-γ expression in SLE patients (r=0.588, P<0.001), and are shown in **Figure 2**. However, in healthy controls, there is no association between IL-12 and IFN-γ (r=0.227, P=0.139). These results were shown in **Table 2**, suggesting a relationship between IL-12 and IFN-γ expression in SLE patients.

ROC analysis

Table 3 lists the areas under ROC curves (AUC) of plasma IL-12 and IFN-γ. IL-12 had a higher AUC (0.756 (95% CI 0.654, 0.858), P<0.001). Meanwhile, the AUC for IFN-γ was 0.653 (95% CI 0.538, 0.769) (P=0.013). This indicated that IL-12 is a better predicted cytokine compared with IFN-γ. The AUCs for IL-12 and IFN-γ are shown in **Figure 3**.

ROC analysis determined that the IL-12 cut off value of 15.635 pg/ml yielded a sensitivity of 81.82%, specificity of 68.18%, PPV of 72.00%, NPV of 78.95% in predicting SLE. In addition, a cut-off value of 272.460 pg/ml for IFN-γ also resulted in a sensitivity, specificity, PPV and NPV of 63.64, 65.91, 65.12 and 64.45% for prediction of SLE respectively.

Discussion

The current view of SLE is that it is the most heterogeneous chronic disease with evolving immunopathologic processes [23]. Currently, there is a high level of interest in the identification and validation of biomarkers that precisely predicting for SLE. Previous researches had reported that Th1 cytokines, were abnormal in SLE [24-26]. Present data suggest an involvement of Th1 cytokines (IL-12 and IFN-γ) in pathogenesis of SLE.

IL-12, produced by macrophage and dendritic cells (DC), is a pro-inflammatory cytokine, induces the differentiation of Th1 cells, and links innate resistance and adaptive immunity [10, 27]. IFN-γ, principally produced principally by T cells, CD4+ as well as CD8+, and natural killer cells, links innate and acquired response of macrophages [28, 29]. Our present data showed that inflammatory cytokines (IL-12 and IFN-γ levels) in SLE patients were higher than the healthy controls, which are in agreement with some previous reports [14, 17, 30-34]. Previous experimental studies had shown that IFN-γ can accelerate disease of SLE, while anti-IFN-γ antibody and soluble recombinant IFN-γR (sIFNR) can delay onset of the disease [35, 36]. Thus, overexpression of IL-12 and IFN-γ causes further inflammation and tissue injury and contributes to the immunopathogenesis of SLE.

Table 2. Levels of Cytokines in Patients with SLE and in Healthy Controls (mean ± SD) (pg/ml)

Cytokines	SLE patients (n=44)	Healthy controls (n=44)	t	*P*
IL-12	19.6464 ± 5.6560	13.2792 ± 6.2767	4.999	<0.001
IFN-γ	293.7754 ± 113.7009	241.9844 ± 81.4744	2.456	0.016
r	0.588	0.227		
P	<0.001	0.139		

SLE, systemic lupus erythematosus.

Table 3. Area under the curve (AUC) of receiver operating characteristic (ROC) curves for SLE prediction

Model	IL-12	IFN-γ
Area Under the ROC Curve	0.756	0.653
95% CI	0.654-0.858	0.538-0.769
P	<0.001	0.013
Cut-off value	15.635	272.46
Sensitivity (%)	81.82	63.64
Specificity (%)	68.18	65.91
PPV (%)	71.9993	65.12351
NPV (%)	78.9486	64.44983
Youden index J	0.5000	0.2955

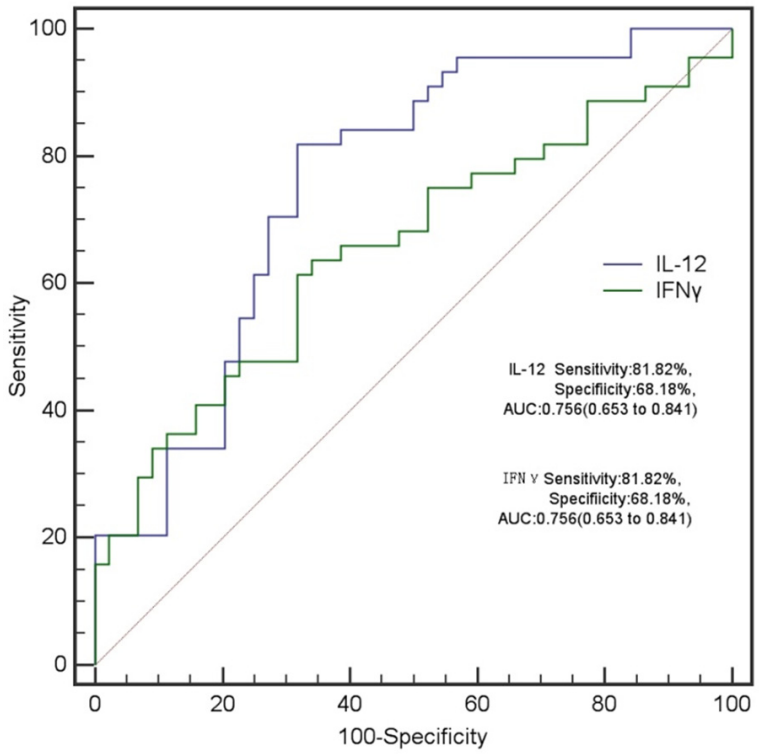

Figure 3. Receiver operating characteristic (ROC) curves and area under the curve (AUC) of receiver ROC curves for SLE.

The present study showed a significant correlation between IL-12 and IFN-γ in SLE patients (r=0.588, P<0.001), but no correlation with healthy controls (P=0.139). IL-12 can induce the production of IFN-γ [10, 27, 37]. This result suggested the increased production of IL-12 was closely in parallel with the increased IFN-γ in SLE. That is, with increased production of IL-12, the increased level of IFN-γ excessively accumulates to the inflammatory site in SLE patients.

From the above analysis, we thought that a strong correlation between SLE and the two cytokines can be established. Now that the two cytokines could be as biomarkers of the SLE, then, we performed ROC curve analysis for predicting SLE with IL-12 and IFN-γ expressions. The AUCs of IL-12 and IFN-γ for predicting SLE were 0.756 (P<0.001; A specificity of 81.82%; A sensitivity of 68.18%) and 0.653 (P= 0.013; A specificity of 63.64%; A sensitivity of 65.91%) respectively. This indicated that IL-12 and IFN-γ can be considered as two well-recognized risk factors in SLE. It can be assisted in SLE prediction once plasma levels of the two cytokines in patients exceeding cut off values of IL-12 (15.635 pg/ml) and IFN-γ (272.46 pg/ml).

Our study has some limitations. Firstly, this is a retrospective case-control study; therefore, subject selection bias cannot be ignored. Secondly, the current results are derived from a sample of moderate size and that the present study cannot establish any causative connection; therefore, further studies with bigger samples are needed to verify the current findings, and modify the cut off values of IL-12 and IFN-γ for predicting SLE.

Finally, it should be noted that the subjects in this study were enrolled from Han Chinese, thus conclusions of this study should be carefully extended to the SLE patients enrolled from other nationalities and ethnics.

Conclusion

To summarize, our study indicates that the elevated levels of IL-12 and IFN-γ in the plasma of patients may be closely associated with SLE pathogenesis. The elevated plasma IL-12 and IFN-γ could serve as predictors of diagnosis in Han Chinese SLE.

Disclosure of conflict of interest

None.

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