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Original Article

Circulating Interleukin-18 Level in Systemic Lupus Erythematosus

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Objective. This study aimed to evaluate the relationship between circulating interleukin (IL)-18 levels and systemic lupus erythematosus (SLE) and establish a correlation between plasma/serum IL-18 levels and SLE activity. **Methods.** We performed a meta-analysis comparing plasma/serum IL-18 levels in patients with SLE to controls by using fixed or random effects model based on the heterogeneity. **Results.** Sixteen studies with 659 SLE patients and 502 controls were included in this meta-analysis. Meta-analysis showed that IL-18 levels were significantly higher in the SLE group (standardized mean difference = 1.556, 95% confidence interval = 1.087 ~ 2.024, $p < 0.001$). Stratifying by ethnicity showed that IL-18 levels were significantly elevated in the SLE groups of European, Asian, and Arab populations. Stratification by adjustment for age and/or sex revealed a significantly higher IL-18 level in the SLE group, independently of the adjustment. Subgroup analysis by sample size showed significantly higher IL-18 levels in the SLE group for both large sample ($n \geq 50$) and small sample ($n < 50$) subgroups. Subgroup analysis by data type showed significantly higher IL-18 levels in the SLE group for both original and calculated data populations. **Conclusion.** This meta-analysis demonstrated that circulating IL-18 levels are higher in patients with SLE. **(J Rheum Dis 2020;27: 110-115)**

Key Words. Interleukin-18, Lupus erythematosus, systemic, Association

INTRODUCTION

Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease characterized by B cell hyperactivity, high levels of autoantibody production, immune-complex deposition, and multiple organ damage [1]. In SLE, the accumulation of self-antigens due to impaired clearance facilitates autoimmune responses and subsequent inflammation with high levels of inflammatory cytokines [2].

Interleukin-18 (IL-18) was initially described as a factor that enhanced interferon-gamma (IFN-γ) in mouse spleen cells [3] and IL-18 plays a key role in autoimmune diseases by controlling either T-helper 1 (Th1) or T-helper 2 (Th2) immune responses [4]. IL-18 is produced by various cell types including Kupffer cells, activated macrophages, keratinocytes, intestinal epithelial cells, osteoblasts, and adrenal cortex cells. IL-18 expression induces the production of tumor necrosis factor-α, granulocyte/macrophage colony-stimulating factor, and IFN-γ, and increases the cytotoxic effects of NK and T cells in SLE [5].

IL-18 may play a key role in the pathogenesis of SLE, however studies comparing the levels of circulating IL-18 in SLE patients and healthy controls, and studies of the relationship between IL-18 levels and SLE activity have shown mixed results. These disparities may be due to small sample sizes, low statistical power, and/or clinical heterogeneity [5-20]. In order to overcome the limitations of individual studies and resolve inconsistencies, we performed a meta-analysis. The present study aimed to determine plasma/serum IL-18 levels in SLE patients compared to those in healthy controls.

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MATERIALS AND METHODS

Identification of eligible studies and data extraction

We performed a literature search for studies that examined IL-18 status in SLE patients and controls, and the relationship between circulating (serum or plasma) IL-18 levels and SLE activity. PubMed, EMBASE, and Cochrane databases were searched to identify all available articles up to July 2019. The following keywords and subject terms were used in the search: "IL-18," "serum OR plasma OR level OR activity," "systemic lupus erythematosus," and "SLE". All references cited were also reviewed to identify additional studies not covered by the above-mentioned electronic databases. Studies were considered eligible based on the following inclusion criteria: (1) they were case-control or cross-sectional studies, and (2) they provided data on IL-18 levels in case and control groups. Studies were excluded if: (1) they contained overlapping or insufficient data, or (2) they were reviews or case reports. Data on methods and results were extracted from the original studies by two independent reviewers. Any discrepancies between reviewers were resolved by consensus, and the meta-analysis was conducted in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines [21]. The following information was extracted from each study: primary author, year of publication, country, ethnicity, number of participants, and mean and standard deviation (SD) of IL-18 levels. When the data given represented medians, interquartile ranges, or ranges, we computed the mean and SD using previously described formulae [22,23].

Evaluation of statistical associations

We performed a meta-analysis examining the relationship between IL-18 levels and SLE. For data continuity, results were presented as standardized mean differences (SMDs) or as correlation coefficients and 95% confidence intervals (CIs). SMDs were calculated by dividing the mean difference between two groups by the pooled SD and were used when different scales were utilized to measure the same concept. This measure compares case and control arms in terms of standardized scores. SMD magnitudes were categorized as follows: 0.2～0.5, small effect; 0.5～0.8, medium effect; ≥0.8, large effect [24]. We also assessed within-study and between-study variability and heterogeneity using Cochran's Q-statistics [25]. The heterogeneity test was used to assess the null hypothesis that all studies were evaluating the same effect. When the Q-statistic indicated significant ($p < 0.10$) heterogeneity across studies, a random effects model was used in the meta-analysis [26]. If significant heterogeneity was not detected, a fixed effects model was used. The fixed effects model assumed that all studies estimated the same underlying effect, and therefore considered within-study variation only [25]. We quantified the effect of heterogeneity using $I^2=100\%\times(Q-df)/Q$ [27], where I^2 measured the degree of inconsistency between studies and determined whether the percentage total variation across studies was due to heterogeneity rather than chance. I^2 values ranged between 0% and 100%; I^2 values of 25%, 50%, and 75% were referred to as low, moderate, and high estimates, respectively [27]. Statistical manipulations were performed using the Comprehensive Meta-Analysis computer program (Biostat Inc., Englewood, NJ, USA).

Evaluation of heterogeneity, sensitivity test, and publication bias

To examine potential sources of heterogeneity observed in the meta-analysis, a meta-regression analysis was performed using the following variables: ethnicity, data type, adjustment for age and/or sex, and sample size. A sensitivity test to assess the influence of each individual study on the pooled SMR was performed by omitting each study individually. We evaluated publication bias using funnel plots and Egger's linear regression test [28], which measured funnel plot asymmetry using a natural logarithm scale of SMRs. When asymmetry was indicated, we used the "trim and fill" method to adjust summary estimates for the observed bias [29]. This method removes small studies until funnel plot symmetry is achieved by recalculating the center of the funnel before the removed studies are replaced with their missing mirror-image counterparts; a revised summary estimate was then calculated using all original studies and hypothetical "filled" studies.

RESULTS

Studies included in the meta-analysis

We identified 134 studies using electronic and manual search methods and 21 of these were selected for full-text review based on the title and abstract. We excluded 5 studies because they had no data on IL-18 levels, or contained duplicate data. Ultimately, 16 articles met the inclusion criteria [5-20], and they were considered in the

meta-analysis, which comprised 659 SLE patients and 502 controls (Table 1). Table 1 summarizes selected characteristics of these studies that were related to the association between IL-18 levels and SLE.

Meta-analysis comparing the circulating IL-18 levels between SLE patients and controls

IL-18 levels were significantly higher in the SLE group than in the control group (SMD=1.556, 95% CI=1.087~2.024, p<0.001) (Table 2, Figure 1). Stratifying data by ethnicity showed a significantly elevated IL-18 level in the SLE group in European, Asian, and Arab populations (Table 2). Stratification by adjustment for age and/or sex revealed a significantly higher IL-18 level in the SLE group, independently of the adjustment (Table 2). Subgroup analysis by sample size showed significantly higher IL-18

Table 1. Characteristics of individual studies included in the meta-analysis

Author	Country	Ethnicity	Cohort size (n)		IL-18 levels (pg/mL)		Statistical findings		
			Cases	Controls	Cases	Controls	SMD	Magnitude*	p-value
Sigdel et al., 2016 [6]	China	Asian	32	24	76.12	11.67	3.291	Large	<0.001
Fouad et al., 2014 [7]	Egypt	Arab	50	50	296.90	112.90	3.504	Large	<0.001
Aghdashi et al., 2013 [8]	Iran	Arab	25	25	281.15	85.12	4.284	Large	<0.001
Koenig et al., 2012 [9]	Switzerland	European	12	14	328.66	67.41	0.894	Large	0.030
Hermansen et al., 2012 [10]	Denmark	European	26	10	59.00	11.00	1.233	Large	0.002
Shimizu et al., 2012 [11]	Japan	Asian	12	32	570.00	244.00	2.254	Large	<0.001
Sahebari et al., 2012 [12]	Iran	Arab	114	50	370.28	84.91	0.710	Medium	<0.001
Xu et al.-1, 2007 [13]	Singapore	Asian	48	47	217.30	136.70	0.524	Medium	0.012
Xu et al.-2, 2007 [13]	Singapore	Asian	22	45	214.20	143.70	0.593	Medium	0.025
Xu et al.-3, 2007 [13]	Singapore	Asian	6	21	75.30	65.90	0.186	Small	0.688
Liang et al., 2006 [14]	China	Asian	16	11	767.00	238.90	4.752	Large	<0.001
Tso et al., 2006 [15]	Taiwan	Asian	70	34	254.34	189.66	0.581	Medium	0.006
Lit et al., 2006 [16]	Hong Kong	Asian	40	40	250.00	171.33	0.805	Large	0.001
Mosaad et al., 2003 [17]	Egypt	Arab	32	21	2343.46	24.41	1.842	Large	<0.001
Liu et al., 2012 [18]	China	Asian	46	20	146.00	48.00	1.112	Large	<0.001
Amerio et al., 2002 [5]	Italy	European	20	20	278.20	185.00	0.837	Large	0.011
Robak et al., 2002 [19]	Poland	European	52	20	753.30	267.30	0.864	Large	0.002
Wong et al., 2000 [20]	Hong Kong	Asian	36	18	368.70	141.10	1.372	Large	<0.001

IL-18: interleukin -18, SMD: standard mean difference. *Magnitude of Cohen's d effect size, where 0.2 to 0.5 is a small effect, 0.5 to 0.8 is a medium effect, and ≥0.8 is a large effect.

Table 2. Meta-analysis of the association between circulating IL-18 levels and SLE

Groups	Population	No. of studies	Test of association			Test of heterogeneity		
			SMD†	95% CI	p-value	Model	p-value	I^2
All	Overall	18	1.556	1.087~2.024	<0.001	R	<0.001	91.0
Ethnicity	European	4	0.929	0.596~1.261	<0.001	F	0.868	0
	Asian	10	1.397	0.828~1.966	<0.001	R	<0.001	88.9
	Arab	4	2.549	0.916~4.183	0.002	R	<0.001	96.5
Adjustment	Yes*	11	1.701	1.062~2.339	<0.001	R	<0.001	92.7
	NA	7	0.898	0.663~1.134	<0.001	R	<0.001	86.4
Sample size	Number ≥50	12	1.560	0.992~2.128	<0.001	R	<0.001	92.8
	Number <50	6	1.566	0.647~2.486	0.001	R	<0.001	85.6
Data type	Original	13	1.652	1.040~2.264	<0.001	R	<0.001	92.1
	Calculated	5	1.343	0.578~2.108	0.001	R	<0.001	88.5

IL-18: interleukin-18, SLE: systemic lupus erythematosus, SMD: standard mean difference, CI: confidence interval, NA: not available, F: fixed effects model, R: random effects model. *Adjustment or non-significance for age- and/or sex. †Magnitude of Cohen's d effect size (SMD), where 0.2 to 0.5 is a small effect, 0.5 to 0.8 is a medium effect, and ≥0.8 is a large effect.

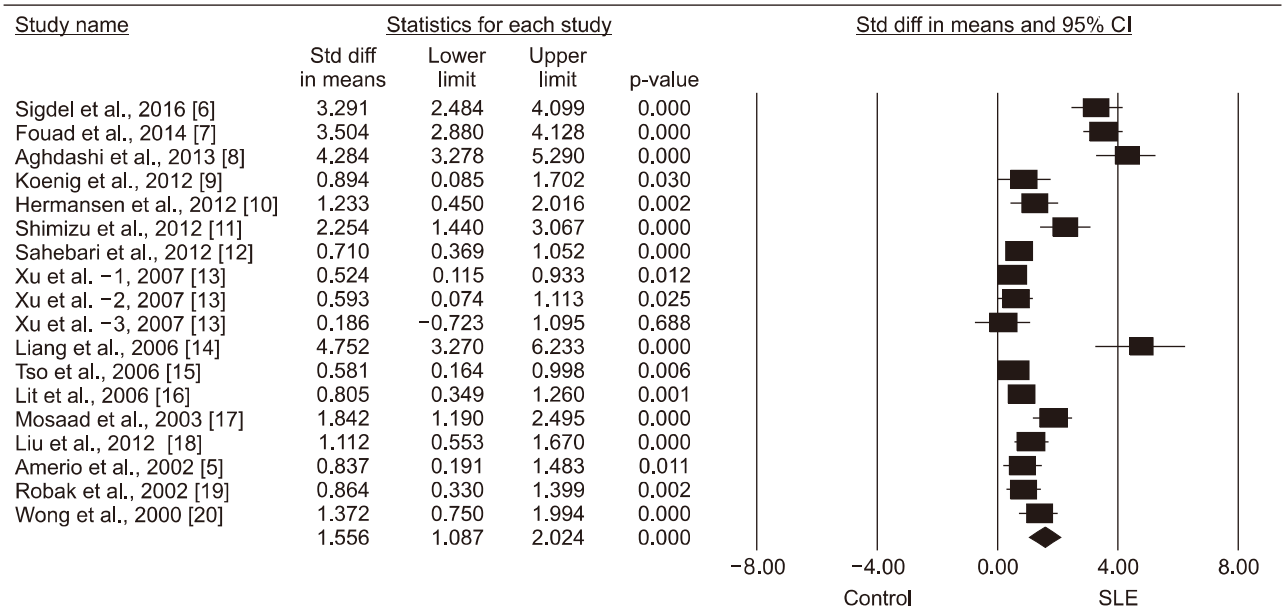

Figure 1. Meta-analysis of relationship of interleukin-18 level with systemic lupus erythematosus (SLE). Std diff: standardized difference, CI: confidence interval.

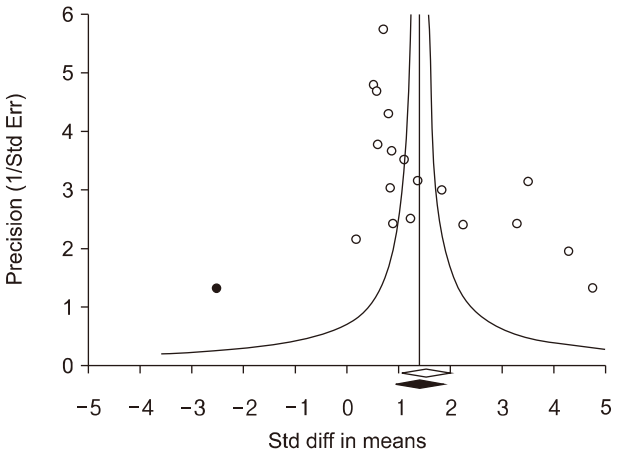

Figure 2. Funnel plot investigating the relation of interleukin-18 with systemic lupus erythematosus (Egger regression p-value=0.003). Filled circles reflect studies showing publication bias. Diamonds at the bottom of the figure display estimates of summary effect before (open) and after (filled) adjustment of publishing bias. Std diff: standardized difference, Std err: standardized error.

levels in the SLE group for both large sample ($n \geq 50$) and small sample ($n < 50$) subgroups (Table 2). Subgroup analysis by data type showed significantly higher IL-18 levels in the SLE group for both original and calculated data populations (Table 2).

Heterogeneity, sensitivity testing, and publication bias

Between-study heterogeneity was identified during the meta-analyses of IL-18 status in SLE patients (Table 2). Meta-regression analysis showed that ethnicity (p=0.019) and adjustment (p=0.010), but not data type (p=0.167) or sample size (p=0.420) had significant impacts on heterogeneity in the meta-analyses of IL-18 levels. Sensitivity analysis showed that no individual study significantly affected the pooled odds ration (OR), indicating that the results of this meta-analysis are robust. Publication bias can lead to a disproportionate number of positive studies and poses a problem for meta-analyses. Since Egger's regression test showed evidence of publication bias (Egger's regression test p-values=0.003) and the funnel plot showed asymmetry, the "trim and fill" method was used to adjust for publication bias (Figure 2). However, the SMD that was significant before adjustment remained significant (SMD=1.720, 95% CI=1.225~2.214).

DISCUSSION

In this meta-analysis, we combined the evidence of circulating IL-18 levels in SLE. The 16 included studies represented 659 SLE patients and 502 controls and showed that circulating IL-18 levels were significantly higher in

the SLE group than in the control group. The results from this meta-analysis suggest that circulating IL-18 may play a role in the pathogenesis of SLE.

IL-18 is a pro-inflammatory member of the IL-1 cytokine superfamily that elicits innate and acquired immune responses [30]. IL-18 is expressed in immune cells such as NK cells, dendritic cells, and macrophages. In addition, IL-18 expression is upregulated during SLE, and has been correlated with SLE activity [5]. A previous study also showed that IL-18 can accelerate lupus-like autoimmune disease in MRL/*lpr* mice [31]. Our meta-analysis showed that there was a significant association between high IL-18 levels and SLE, independent of potential confounders such as ethnicity, sample size, or data type. The source of elevated circulating IL-18 in patients with SLE is unclear, however it may be related to a genetic factor, as previous work has shown that overproduction of IL-18 may be due to polymorphisms in regulatory regions of the IL-18 gene, which is located on chromosome 11q22.2–q22.3 [32]. Indeed, three polymorphisms in the IL-18 promoter region (−607 C/A [rs1946518], −137 G/C [rs187238], and −1297 C/T [rs360719]) alter IL-18 promoter activity by changing its transcription activity [33]. In addition, a previous meta-analysis found that these IL-18 polymorphisms are associated with the development of SLE [34]. Because IL-18 is located within the SLE chromosomal susceptibility locus, it is considered that IL-18 is a factor in the genetic susceptibility to SLE [35]. It is not known whether the association found in this meta-analysis is the cause or the consequence of increased IL-18. However, increased IL-18 may be a cause rather than a consequence of disease development, because genetic variation in IL-18 may be associated with higher IL-18 levels.

This meta-analysis has several limitations that should be considered. First, the majority of studies had a small sample size, and only a few studies evaluated the correlation coefficients between IL-18 levels and SLE activity. Thus, the meta-analysis may have been underpowered. Second, the studies included in the meta-analysis were heterogeneous in demographic characteristics and clinical features. Therefore the heterogeneity, confounding factors, and limited clinical information provided by the study population may have affected our results. In addition, these limited data did not permit further analysis, although we performed a sensitivity test and a meta-regression analysis. Despite these limitations, this meta-analysis also has several strengths. To the best of our knowledge, our meta-analysis is the first to provide combined evidence for IL-18 status in SLE patients. In addition, previous studies used population sizes that ranged from 6 to 114, whereas we presented a pooled analysis of 659 patients. Similarly, by pooling the results of independent analyses, our analysis of the relationship between IL-18 levels and SLE had increased statistical power and resolution and therefore greater accuracy in comparison to previous individual studies.

CONCLUSION

In conclusion, our meta-analysis demonstrated that circulating IL-18 levels were significantly higher in patients with SLE than in controls, regardless of ethnicity, sample size, and data type evaluated, and that a significantly positive correlation existed between IL-18 level and SLE activity. Thus, our meta-analysis suggests that IL-18 plays a critical role in SLE, though further studies are necessary to elucidate the mechanism through which IL-18 levels directly contribute to the pathogenesis of SLE.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

AUTHOR CONTRIBUTIONS

Y.H.L. was involved in conception and design of study, acquisition of data, analysis and/or interpretation of data, drafting the manuscript, revising the manuscript critically for important intellectual content. G.G.S. was involved in conception and design of study, analysis and/or interpretation of data, drafting the manuscript

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