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 ≥ 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.

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.
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 Cochrane’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 \( I^2 = \frac{100\times(Q-df)}{Q} \) [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).

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

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

| Author              | Country | Ethnicity | Cohort size (n) | IL-18 levels (pg/mL) | Statistical findings |
|---------------------|---------|-----------|-----------------|----------------------|----------------------|
| Sigdel et al., 2016 | China   | Asian     | 32 24           | 76.12 24.67          | SMD=3.291 Large <0.001 |
| Fouad et al., 2014  | Egypt   | Arab      | 50 50           | 296.90 112.90        | SMD=3.504 Large <0.001 |
| Aghdashi et al., 2013 | Iran    | Arab      | 25 25           | 281.15 85.12         | SMD=4.284 Large <0.001 |
| Koenig et al., 2012 | Switzerland | European | 12 14           | 328.66 67.41         | SMD=0.894 Large 0.030 |
| Hermansen et al., 2012 | Denmark  | European  | 26 10           | 59.00 11.00          | SMD=1.233 Large 0.002 |
| Shimizu et al., 2012 | Japan   | Asian     | 12 32           | 570.00 244.00        | SMD=2.254 Large <0.001 |
| Sahebari et al., 2012 | Iran    | Arab      | 114 50          | 370.28 84.91         | SMD=0.710 Medium <0.001 |
| Xu et al.-1, 2007   | Singapore | Asian     | 48 47           | 217.30 136.70        | SMD=0.524 Medium 0.012 |
| Xu et al.-2, 2007   | Singapore | Asian     | 22 45           | 214.20 143.70        | SMD=0.593 Medium 0.025 |
| Xu et al.-3, 2007   | Singapore | Asian     | 6 21            | 75.30 65.90          | SMD=0.186 Small 0.688 |
| Liang et al., 2006  | China   | Asian     | 16 11           | 767.00 238.90        | SMD=4.752 Large <0.001 |
| Tso et al., 2006    | Taiwan  | Asian     | 70 34           | 254.34 189.66        | SMD=0.581 Medium 0.006 |
| Lit et al., 2006    | Hong Kong | Asian    | 40 40           | 250.00 171.33        | SMD=0.805 Large 0.001 |
| Mosaad et al., 2003 | Egypt   | Arab      | 32 21           | 2343.46 24.41        | SMD=1.842 Large <0.001 |
| Liu et al., 2012    | China   | Asian     | 46 20           | 146.00 48.00         | SMD=1.112 Large <0.001 |
| Amerio et al., 2002 | Italy   | European  | 20 20           | 278.20 185.00        | SMD=0.837 Large 0.011 |
| Robak et al., 2002  | Poland  | European  | 52 20           | 753.30 267.30        | SMD=0.864 Large 0.002 |
| Wong et al., 2000   | Hong Kong | Asian    | 36 18           | 368.70 141.10        | SMD=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.

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**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 I²     |
| 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.
levels in the SLE group for both large sample (n≥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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