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Original Article

Umbilical cord mesenchymal stem cell-derived secretome as a potential treatment for systemic lupus erythematosus: A double-blind randomized controlled trial

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Abstract

Umbilical cord mesenchymal stem cell-derived (UCMSC-derived) secretome is anti-apoptotic, anti-inflammatory, antifibrotic, angiogenic, and tissue-regenerating. Thus, it may treat systemic lupus erythematosus (SLE). The aim of this study was to investigate the impact of the UCMSC-derived secretome on SLE patients' disease activity, using Mexican systemic lupus erythematosus disease activity index (MEX-SLEDAI) score, complement (C3 and C4) levels, tumor necrosis factor-alpha (TNF-α), anti-double-stranded DNA (anti-dsDNA), and interleukin-6 (IL-6) levels. This double-blind randomized controlled trial investigated the efficacy and safety of UCMSC-derived secretome in SLE patients with moderate disease activity. A total of 29 female patients were randomized into two groups to receive weekly 1.5 cc intramuscular injections of UCMSC-derived secretome or placebo (0.9% NaCl) for six weeks. Disease activity was assessed using the MEX-SLEDAI score, C3 and C4 levels, pro-inflammatory cytokines (IL-6 and TNF-α), and anti-dsDNA antibodies at baseline, Day 22, and Day 43. Results showed a significant reduction in MEX-SLEDAI scores in the secretome group compared to the placebo group ($p<0.05$). Complement C3 levels significantly increased in the secretome group on Day 43, indicating improved immune homeostasis, while C4 levels did not show significant differences between groups. IL-6 and TNF-α levels showed decreasing trends in the secretome group. Anti-dsDNA levels exhibited a decreasing trend in the secretome group, though not statistically significant. Importantly, no severe adverse events were observed, underscoring the safety of the intervention. UCMSC-derived secretome demonstrated immunomodulatory and anti-inflammatory effects, reducing disease activity in SLE patients. These findings suggest its potential as a safe and effective adjunct therapy for SLE, although further studies with larger sample sizes and extended follow-up periods are needed to validate these results.

Keywords: Systemic lupus erythematosus, lupus, MEX-SLEDAI, secretome, mesenchymal stem cell

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by autoantibodies that affect various organ systems [1]. SLE is a chronic autoimmune disease with a global

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prevalence ranging from 20 to 150 cases per 100,000 individuals [2]. It significantly affects quality of life and carries high morbidity and mortality rates despite advancements in treatment [2]. Although there is no definitive cure for SLE, current treatment strategies focus on non-specific anti-inflammatory and immunosuppressive agents to manage immunological disorders, including antimalarial drugs, glucocorticoids, non-corticosteroid immunosuppressants, and targeted therapies [3]. Current therapeutic strategies, including immunosuppressive agents and biologics, have limitations such as low remission rates, significant side effects, and a lack of specificity. These challenges highlight the pressing need for novel and effective therapies for SLE [2,3].

The pathogenesis of SLE involves complex interactions between genetic and environmental factors, leading to immune dysregulation and the production of autoantibodies. Key pathogenic mechanisms include the loss of immune tolerance, activation of autoreactive T and B cells, and the formation of immune complexes that contribute to tissue damage [4,5]. Activation of B and T cells triggers the release of autoantibodies such as anti-double-stranded DNA (anti-dsDNA), which are specific to SLE, particularly lupus nephritis. The excessive use of complement C3 and C4 for apoptotic body clearance results in decreased C3 and C4 levels in the blood. Additionally, the activation of several pro-inflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), further exacerbates the disease [5]. Common complications, such as lupus nephritis, which occurs in approximately 50% of SLE patients, and vascular inflammation, underscore the need for improved management strategies. Anti-dsDNA antibodies and complement markers, such as C3 and C4, are well-established biomarkers for disease diagnosis and activity assessment [6].

Mesenchymal stem cell (MSC) therapy has emerged as a promising approach for autoimmune diseases due to its immunomodulatory and anti-inflammatory properties [7,8]. The umbilical cord mesenchymal stem cell-derived (UCMSC-derived) secretome, which consists of bioactive molecules such as cytokines, growth factors, and extracellular vesicles, offers advantages over cell-based therapies, including ease of administration, cost-effectiveness, and reduced immunogenicity [9]. UCMSC-derived secretome can suppress T cell activation, stimulate Treg cell differentiation, inhibit NK cells, and prevent dendritic cell maturation, thereby reducing B cell expansion and autoantibody production. Preclinical studies have demonstrated the potential of mesenchymal stem cell (MSC) secretome to modulate immune responses and promote tissue regeneration [7,8]. However, clinical data on their application in SLE remains limited [10].

The aim of this study was to evaluate the efficacy and safety of UCMSC-derived secretome as an adjunct therapy for SLE. Its impact on disease activity was measured by the Mexican systemic lupus erythematosus disease activity index (MEX-SLEDAI) score, as well as key biomarkers such as complement levels (C3 and C4), pro-inflammatory cytokines (IL-6 and TNF-α), and anti-dsDNA antibodies. By addressing the knowledge gap in MSC-derived secretome applications for SLE, this study sought to contribute to the development of innovative therapeutic strategies for this challenging autoimmune condition [10,11].

Methods

Study design, setting, and sampling

A double-blind, randomized, placebo-controlled trial was conducted at the Rheumatology Polyclinic of Dr. Moewardi General Hospital, Surakarta, Indonesia, from May 2023 to June 2023. This study was registered on ClinicalTrials.gov (ID NCT05921058; registration date: June 27, 2023). The sample size was calculated using the OpenEpi website (https://www.openepi.com /SampleSize/SSMean.htm), with a minimum requirement of 28 subjects. To account for potential dropouts or ineligibility, a recruitment target of 36 subjects was set, including eight reserves. Participants were randomly assigned to either the treatment group, which received UCMSC-derived secretome, or the control group, which was administered a placebo in the form of 0.9% sodium chloride (NaCl). Outcome measures included the MEX-SLEDAI score, complement levels (C3 and C4), pro-inflammatory cytokines (IL-6 and TNF-α), and anti-dsDNA antibody levels. Measurements were conducted at baseline, mid-intervention (Day 22), and post-intervention (Day 43).

Patient's criteria and randomization

Patients included in the study were women aged 18–75 years, diagnosed with SLE based on American College of Rheumatology and European League Against Rheumatism (ACR/EULAR) 2019 criteria, have moderate degree of activity, meeting at least one of the following criteria: alopecia with scalp inflammation, arthritis, fever, hepatitis, pleurisy, pericarditis, skin rash up to 9–18% body surface area, skin vasculitis ≤18% body surface area, platelets 20,000–50,000/mm^3, systemic lupus erythematosus disease activity index (SLEDAI) score of 6–12 or MEX-SLEDAI assessment score of 6–9, and had provided informed consent. Exclusion criteria included pregnancy, cancer, male sex, use of biologic agents such as anti-IL-6 or TNF-α, severe disease activity, and history of allergies [16]. Dropout criteria included loss to follow-up, withdrawal of consent, development of severe adverse events, or non-adherence to the intervention protocol.

Eligible patients were randomized in a 1:1 ratio to either the intervention group, receiving UCMSC-derived secretome, or the control group, receiving a placebo (NaCl 0.9%). Randomization was conducted using a computer-generated random number sequence by an independent team to ensure allocation concealment and minimize selection bias.

Intervention

The UCMSC-derived secretome utilized in this study was produced by PT. Bifarma Adiluhung, Jakarta, Indonesia, an accredited good manufacturing practices (GMP) facility certified by the Indonesian Food and Drug Authority (Certification Number: PWS.01.04.1.3.333.09.21-0082). The manufacturing process was authorized by the Ministry of Health, Indonesia (License No: 11/1/10/KES/PMDN/2018) for stem cell-based products. The secretome was formulated as a sterile, aqueous solution devoid of antibiotics, preservatives, or red phenol to ensure compatibility and safety for clinical use.

An independent team was established, comprising a randomization team, a preparation team, and an execution team. The patients received the intervention at the rheumatology clinic on a predetermined schedule. Before treatment, each subject was reassessed for their initial condition, MEX-SLEDAI score, and blood sample collection. The randomization team assigned a unique code to each patient and provided the code to the preparation team. The preparation team prepared UCMSC-derived secretome and 0.9% NaCl placebo, each in 1.5 mL using identical 3 mL syringes. Each syringe was sealed with black opaque tape and labeled with the unique code to ensure blinding. The preparation team then handed the syringes to the execution team, who administered the intramuscular injections aseptically into the right arm of each patient. Blinding was maintained across all study processes to ensure unbiased outcomes. Patients were observed in the clinic for one hour after each injection to monitor for any adverse events. Injections were administered weekly for six weeks. All participants continued their routine therapy according to established guidelines throughout the study.

Data collection

Patients were closely monitored throughout the study, with routine laboratory evaluations conducted at predefined intervals following each intervention. Baseline data included age, standard therapy received, duration of previous treatment, and nutritional status, which were collected on Day 0 (before treatment). Disease activity assessments, including MEX-SLEDAI scores, and laboratory measurements of C3, C4, IL-6, and TNF-α levels, were conducted on Days 0 (pre-intervention), 22 (middle of the intervention), and 43 (end of the intervention).

The MEX-SLEDAI score was calculated following the Indonesian Rheumatology Association's recommendation [12]. The assessment included 12 domains: neurological disorders (8 points), renal disorders (6 points), vasculitis (4 points), hemolysis (3 points), myositis (3 points), arthritis (2 points), mucocutaneous disorders (2 points), fatigue (1 point), fever (1 point), serositis (1 point), and lymphopenia/leukopenia (1 point). Scores of 0 indicated remission, 1–5 indicated low disease activity, 6–9 indicated moderate disease activity, and 10–15 indicated severe disease activity [12].

Laboratory analyses were performed at the Clinical Pathology Laboratory of Dr. Moewardi General Hospital, Surakarta, Indonesia. Blood samples (6 mL) were collected, and the complement C3 and C4 levels were measured using the Tina-quant Complement C3c ver.2 and Tina-quant Complement C4 ver.2 assays (Roche Diagnostics, New York, USA), with results

reported in mg/dL. Serum IL-6 levels were determined using the Elecsys IL-6 assay (Roche Diagnostics, New York, USA), with results reported in pg/mL. TNF-α levels were quantified using the Invitrogen TNF-α (Total) Human ELISA Kit (Thermo Fisher Scientific, New York, USA), also reported in pg/mL. Anti-dsDNA antibodies were measured using the ds-DNA Ab IgG ELISA (Demeditec Diagnostics, New York, USA).

Adverse events were recorded using an adverse event report form. Acute adverse events were evaluated 1 hour and 24 hours post-injection, with patients instructed to report any events through a dedicated hotline. Adverse events reported during subsequent visits for weekly injections were also documented. Patients could contact the research team directly through the hotline to report adverse events. Key adverse events monitored included pain or swelling at the injection site, fever, and allergic or anaphylactic reactions.

Statistical analysis

Data normality was tested using the Shapiro-Wilk test. Chi-squared tests were performed to evaluate the difference in the outcomes before and after treatment as appropriate. An unpaired Student t-test was used to assess differences between the control and treatment groups for normally distributed data, while repeated measures analysis of variance (ANOVA) was applied to evaluate variations within groups over time (Day 0, Day 22, and Day 43). For non-normally distributed data, the Mann-Whitney U test was used for between-group comparisons, and the Friedman test was applied for within-group comparisons over time. A $p<0.05$ was considered statistically significant. Correlation analyses were conducted to evaluate the relationship between IL-6, TNF-α, and osteocalcin levels using Pearson's correlation for normally distributed data or Spearman's correlation for non-normally distributed data. All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA).

Results

Characteristics of the patients

A total of 36 subjects consented to participate in the study and underwent an eligibility assessment (**Figure 1**). Of these, 29 subjects met the inclusion criteria, while seven subjects were excluded. Five subjects were excluded due to not meeting the inclusion criteria, and two subjects were not willing to participate. The remaining 29 eligible subjects were randomized using computer-generated random numbers and allocated into two groups: 15 subjects in the treatment group and 14 subjects in the control group. No participants were lost to follow-up or discontinued the intervention throughout the study duration (**Figure 1**).

Comparisons of the characteristics of the patients between groups are presented in **Table 1**. There was no significant difference in the mean age between UCMSC-derived secretome and control groups (37.73±9.38 vs 9.93±11.45 years, respectively) ($p=0.576$). There was also no significant difference in baseline data between UCMSC-derived secretome and control groups regarding the mean scores of disease activity assessments using MEX-SLEDAI (7.333 vs 7.429), complement C3 mean level (1.147 vs 1.267 mg/dL) and C4 mean level (0.228 vs 0.259 mg/dL) (all had $p>0.05$). Baseline data between groups regarding the use of standard medication also showed no significant difference ($p=0.525$), although there were more patients in the control group who used mycophenolic acid (31.0%) compared to 20.7% patients in UCMSC-derived secretome group. The mean duration of treatment was slightly longer in the control group compared to the secretome group, although it was not statistically significant ($p=0.930$). Abnormal nutritional status was significantly prevalent in the secretome group ($p=0.027$) (**Table 1**).

Safety of UCMSC-derived secretome injection

Throughout the study, adverse events were monitored during each patient's visit for intervention or sample collection. Participants also had access to a 24-hour hotline provided by the research team for reporting any concerns. Monitored parameters included fever, pain, diarrhea, cough, signs of allergic reactions, anaphylactic reactions, and abnormalities in vital signs. This comprehensive monitoring approach ensured prompt identification and management of any adverse events, prioritizing patient safety throughout the study.

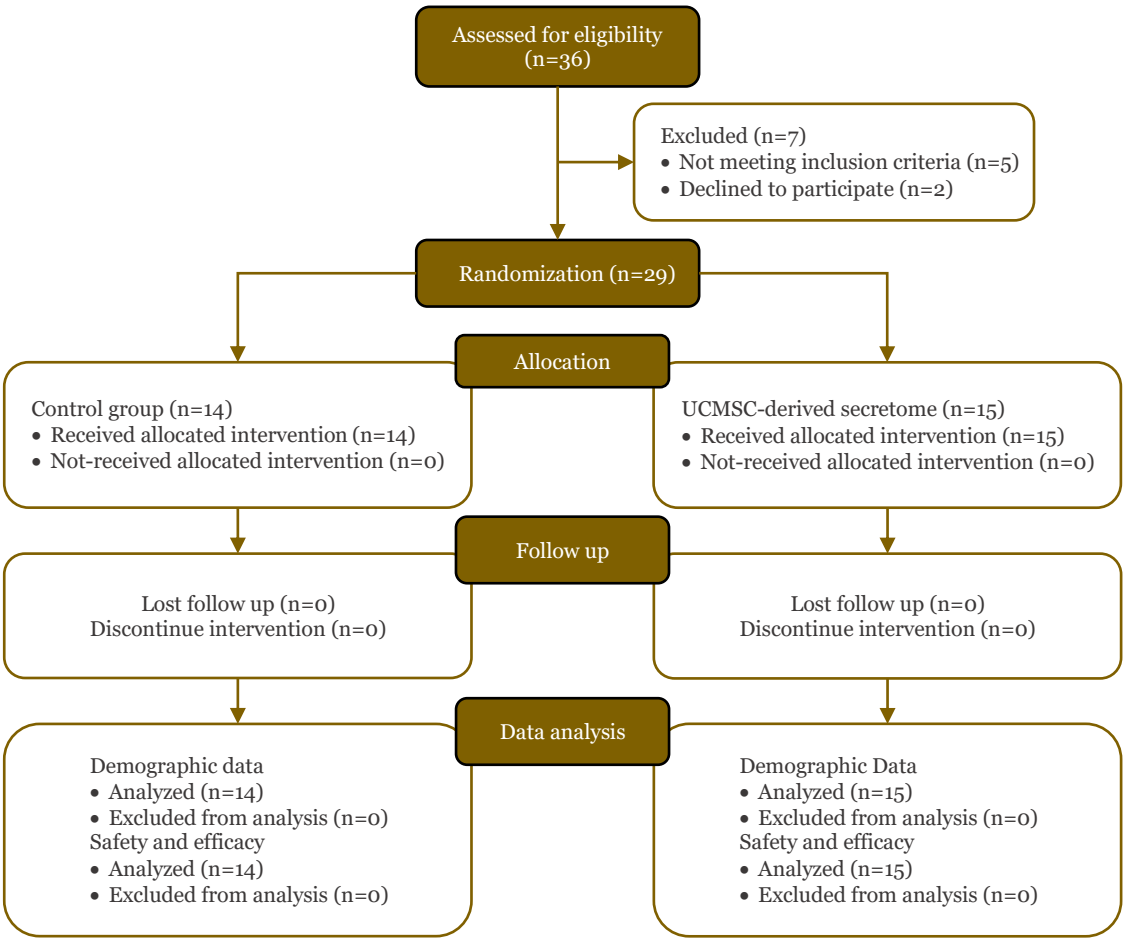

Figure 1. Flow diagram of the patient recruitment and follow-up process. UCMSC: umbilical cord mesenchymal stem cell.

Table 1. Characteristics of the systemic lupus erythematosus (SLE) patients

Characteristics	Frequency (%)		*p*-value
	Control group (n=14)	Secretome group (n=15)	
Age, mean±SD	39.93±11.45	37.73±9.38	0.576[a]
≤40 years	7 (24.1)	7 (24.1)	1.000[b]
>40 years	7 (24.1)	8 (27.6)	
Standard medication			0.525[b]
Leflunomide	2 (6.9)	2 (6.9)	
Mycophenolic acid	9 (31.0)	6 (20.7)	
Cyclosporin	2 (6.9)	5 (17.2)	
Others	1 (3.4)	2 (6.9)	
Duration of treatment, mean±SD (months)	80.57±76.00	79.07±72.45	0.930[a]
Nutritional status			0.027[b*]
Underweight	0 (0)	3 (10.3)	
Normoweight	13 (44.8)	8 (27.6)	
Overweight	1 (3.4)	4 (13.8)	
SLE disease activity parameter			
MEX-SLEDAI, mean±SD	7.429±1.158	7.333±1.047	0.821[c]
Complement C3, mean±SD (mg/dL)	1.267±0.283	1.147±0.235	0.222[a]
Complement C4, mean±SD (mg/dL)	0.259±0.076	0.228±0.076	0.287[a]

MEX-SLEDAI: Mexican systemic lupus erythematosus disease activity index
[a]Analyzed with Mann-Whitney test
[b]Analyzed with Chi-squared test
[c]Analyzed with Mann-Whitney test
*Statistically significant at $p<0.05$

Throughout this investigation, no instances of serious adverse sections or adverse drug events were observed. There were no recorded instances of allergic reactions caused by secretome during the study. Only five reports were documented regarding pain experienced at the site of injection, which subsequently dissipated within a few minutes following the injection.

Efficacy of UCMSC-derived secretome in reducing the MEX-SLEDAI score

Our data indicated that there was significant decrease in the MEX-SLEDAI score in the control group from 7.429±1.158 on Day 0 to 5.143±2.214 on Day 43 accounting for 55.5% decrease compared to baseline (**Table 2**). In UCMSC-derived secretome group, the MEX-SLEDAI score significantly decreased from 7.333±1.047 (Day 0) to 3.267±1.486 (Day 43) (p<0.001) (**Table 2**). However, the UCMSC-derived secretome group had a greater decrease in the MEX-SLEDAI score compared to that in the control group both in the middle (p=0.010) and end of the intervention (p=0.012) (**Table 2**). Furthermore, post-hoc analyses indicated that UCMSC-derived secretome significantly reduced the MEX-SLEDAI score between Day 22 and baseline (p=0.023); and between Day 43 and 0 (p=0.002) but not between Day 43 and Day 22 (p=0.395).

Table 2. Comparisons of MEX-SLEDAI score between secretome and control groups as well as between Day 0, Day 22, and Day 43 of intervention

MEX-SLEDAI score	Control group (n=14)		Secretome group (n=15)		p-value
	Mean	SD	Mean	SD	
Day 0	7.429	1.158	7.333	1.047	0.821[a]
Day 22	5.714	1.816	4.000	1.558	0.010[a*]
Day 43	5.143	2.214	3.267	1.486	0.012[b*]
p-value	0.001[c*]		<0.001[c*]		

MEX-SLEDAI: Mexican systemic lupus erythematosus disease activity index
[a]Analyzed with Mann-Whitney test
[b]Analyzed with independent t-test
[c]Analyzed with Friedman Test
[*]Statistically significant at p<0.05

Effect of secretome on complement C3 levels

The comparison of the complement C3 levels between the secretome and control groups is presented in **Table 3**. The study found that complement C3 levels in the control group decreased from 1.267±0.283 mg/dL on Day 0 to 1.205±0.345 mg/dL on Day 22 and increased to 1.333±0.228 mg/dL or up to 5.2% on Day 43, but the difference was not significant (p=0.163). The C3 levels in the intervention group decreased from 1.147±0.235 to 1.097±0.234 mg/dL on Day 22 and 1.144±0.251 mg/dL on Day 43, but the difference was not significant (p=0.780). This indicated a decrease in complement C3 levels after secretome administration compared to the control group. Additionally, the unpaired difference test showed no significant difference in complement C3 levels between the control and secretome groups on Days 0 and 22 (p=0.338). However, on Day 43, the secretome group showed a significantly higher level of complement C3 (p=0.044), suggesting that secretome administration could increase complement C3 levels (**Table 3**).

Table 3. Comparisons of complement C3 levels between secretome and control groups as well as between Day 0, Day 22, and Day 43 of intervention

Complement C3 levels (mg/dL)	Control group (n=14)		Secretome group (n=15)		p-value
	Mean	SD	Mean	SD	
Day 0	1.267	0.283	1.147	0.235	0.222[a]
Day 22	1.205	0.345	1.097	0.234	0.338[a]
Day 43	1.333	0.228	1.144	0.251	0.044[a*]
p-value	0.163[b]		0.780[b]		

[a]Analyzed with independent Student t-test
[b]Analyzed with repeated ANOVA test
[*]Statistically significant at p<0.05

Effect of secretome on complement C4 levels

The comparisons of complement C4 levels between the secretome and control groups are presented in **Table 4**. In the control group, complement C4 levels were 0.259±0.076 mg/dL on Day 0 and 0.252±0.105 mg/dL on Day 22, representing a 2.5% decrease, which was not significant. By Day 43, the level increased by 14.4% to 0.296±0.114 mg/dL. In the secretome group, levels decreased from 0.228±0.076 mg/dL on Day 0 to 0.212±0.082 mg/dL on Day 22 (7% decrease; p=0.105) and increased to 0.241±0.111 mg/dL by Day 43 (5.8% increase); no

significant change between observation times. Our data indicated no significant differences in complement C4 levels between the secretome and control groups on Days 0 (p=0.287), 22 (p=0.258), or 43 (p=0.205), indicating that secretome administration did not affect complement C4 levels (**Table 4**).

Table 4. Comparisons of complement C4 levels between secretome and control groups as well as between Day 0, Day 22, and Day 43 of intervention

Complement C4 levels (mg/dL)	Control group (n=14)		Secretome group (n=15)		p-value
	Mean	SD	Mean	SD	
Day 0	0.259	0.076	0.228	0.076	0.287[a]
Day 22	0.252	0.105	0.212	0.082	0.258[a]
Day 43	0.296	0.114	0.241	0.111	0.205[a]
p-value	0.311[b]		0.105[b]		

[a]Analyzed with independent Student t-test
[b]Analyzed with repeated Anova test

Effect of secretome on IL-6 levels

The comparisons of the mean IL-6 levels in each group are presented in **Table 5**. Data indicated a significant decrease in IL-6 levels in both the control (p=0.024) and secretome groups (p=0.005). In the control group, IL-6 levels decreased from 67.61±44.42 pg/mL on Day 0 to 49.71±75.35 pg/mL on Day 22, and further to 15.63±13.16 pg/mL on Day 43. In the secretome group, the level of IL-6 reduced from 62.73 pg/mL to 51.45 pg/mL on Day 22 and to 15.57 pg/mL at the end of the intervention. There was no significant difference in the IL-6 reduction between the two groups at each testing point (**Table 5**).

Post-hoc analysis within the secretome group revealed a significant reduction in IL-6 levels between Day 43 and Day 0 (p=0.006). However, no significant reductions were observed between Day 22 and Day 0 (p=1.000) or between Day 43 and Day 22 (p=0.053).

Table 5. Comparisons of IL-6 levels between secretome and control groups as well as between Day 0, Day 22, and Day 43 of intervention

IL-6 levels (pg/mL)	Control group (n=14)		Secretome group (n=15)		p-value
	Mean	SD	Mean	SD	
Day 0	67.61	44.42	62.73	43.06	0.566[a]
Day 22	49.71	75.35	51.45	74.27	0.663[a]
Day 43	15.63	13.16	15.57	15.60	0.485[b]
p-value	0.024[c*]		0.005[c*]		

[a]Analyzed with Mann-Whitney test
[b]Analyzed with independent Student t-test
[c]Analyzed with Friedman test
*Statistically significant at p<0.05

Effect of secretome on TNF-α levels

Comparisons of the mean levels of TNF-α between groups, as well as before and after the intervention, are presented in **Table 6**. Analysis showed no significant decrease in TNF-α levels in the control group after 43 days (p=0.145). In contrast, the secretome group showed a significant decrease, from 24.56 pg/mL (Day 0) to 11.80 pg/mL (Day 22) and 10.87±7.29 pg/mL (Day 43), (p=0.004).

Table 6. Comparisons of TNF-α levels between secretome and control groups as well as between Day 0, Day 22, and Day 43 of intervention

TNF-α levels (pg/mL)	Control group (n=14)		Secretome group (n=15)		p-value
	Mean	SD	Mean	SD	
Day 0	37.39	36.08	24.56	16.53	0.793[a]
Day 22	23.88	28.67	11.80	6.52	0.116[a]
Day 43	11.92	6.43	10.87	7.29	0.631[b]
p-value	0.145[c*]		0.004[c*]		

[a]Analyzed with the Mann-Whitney test
[b]Analyzed with independent Student t-test
[c]Analyzed with Friedman test
*Statistically significant at p<0.05

Post-hoc analysis within the secretome group indicated a significant decrease in TNF-α levels between Day 43 and Day 0 (p=0.006). No significant reductions were observed between Day 22 and Day 0 (p=0.820) or between Day 43 and Day 22 (p=0.053).

Effect of secretome on anti-dsDNA levels

The comparisons of the mean level of anti-dsDNA between the groups, as well as before and after the intervention, are presented in **Table 7**. The level of anti-dsDNA was slightly lower in the secretome group than in the control group but not statistically significant. On Day 43, the level of anti-dsDNA was 17.45±24.44 in the secretome group and 40.52±75.72 in the control group. The Friedman test showed a non-significant decrease in anti-dsDNA levels in both the control and secretome groups.

Table 7. Comparisons of anti-dsDNA levels between secretome and control groups as well as between Day 0, Day 22, and Day 43 of intervention

Anti-dsDNA (pg/L)	Control group (n=14)		Secretome group (n=15)		*p*-value
	Mean	SD	Mean	SD	
Day 0	42.43	76.54	31.06	50.62	0.683[a]
Day 22	45.36	71.78	32.14	46.54	0.310[a]
Day 43	40.52	75.72	17.45	24.44	0.123[a]
p-value	0.135[b]		0.936[b]		

Anti-dsDNA: anti-double-stranded DNA
[a]Analyzed with the Mann-Whitney test
[b]Analyzed with Friedman test

Discussion

This study provides new insights into the therapeutic potential of UCMSC-derived secretome as an adjuvant therapy for SLE. The findings demonstrate its ability to modulate immune pathways, reduce disease activity, and improve key biomarkers, thereby addressing significant gaps in current treatment strategies for SLE. Unlike traditional immunosuppressive therapies, the UCMSC-derived secretome targets multiple immune pathways, including cytokine modulation (IL-6 and TNF-α), complement restoration (C3), and potential reduction in autoantibody production (anti-dsDNA). This multi-target approach offers a more comprehensive strategy for managing SLE. The significant reduction in MEX-SLEDAI scores highlights the efficacy of the secretome in improving clinical outcomes for moderate disease activity, a crucial area often inadequately addressed in existing treatments.

The significant reduction in MEX-SLEDAI scores observed in the secretome group (55.5%) highlights its efficacy in improving clinical outcomes, particularly in patients with moderate disease activity, an area often inadequately addressed by conventional therapies. Notably, the decrease in MEX-SLEDAI scores in the secretome group suggests a shift in disease activity from moderate to mild, further supporting the therapeutic benefit of secretome treatment in this clinical trial [13]. These findings are consistent with existing studies that demonstrated the immunomodulatory and anti-inflammatory properties of mesenchymal stem cell-derived therapies in autoimmune diseases, particularly in terms of enhancing immune tolerance and reducing inflammation [14,15]. The proposed mechanism underlying these effects is illustrated in **Figure 2**, showcasing the complex immune modulation facilitated by UCMSC-derived secretome.

Hematological parameters, such as hemoglobin, lymphocytes, and platelets, are integral components of the MEX-SLEDAI scoring system [16]. In SLE, anemia is a common finding, with patients often exhibiting hemoglobin levels below 12 g/dL. In this study, 51% of subjects demonstrated low hemoglobin levels, aligning with findings from a prior study reporting a prevalence of 70.73% [17]. The etiology of anemia in SLE is multifactorial, encompassing immunological mechanisms, such as antibodies targeting erythropoietin and inflammatory cell infiltration, as well as non-immunological contributors like chronic disease anemia, autoimmune hemolytic anemia, and iron deficiency anemia [18-20]. Thrombocytopenia, another hematological manifestation, was observed in one patient within this study and was attributed to immune-mediated platelet destruction. Notably, platelet counts improved following the

administration of MSC-derived secretome. This aligns with previous research suggesting that autoimmune thrombocytopenia involves mesenchymal stem cell dysfunction, heightening TNF-α levels and exacerbating disease pathology [16,20-22].

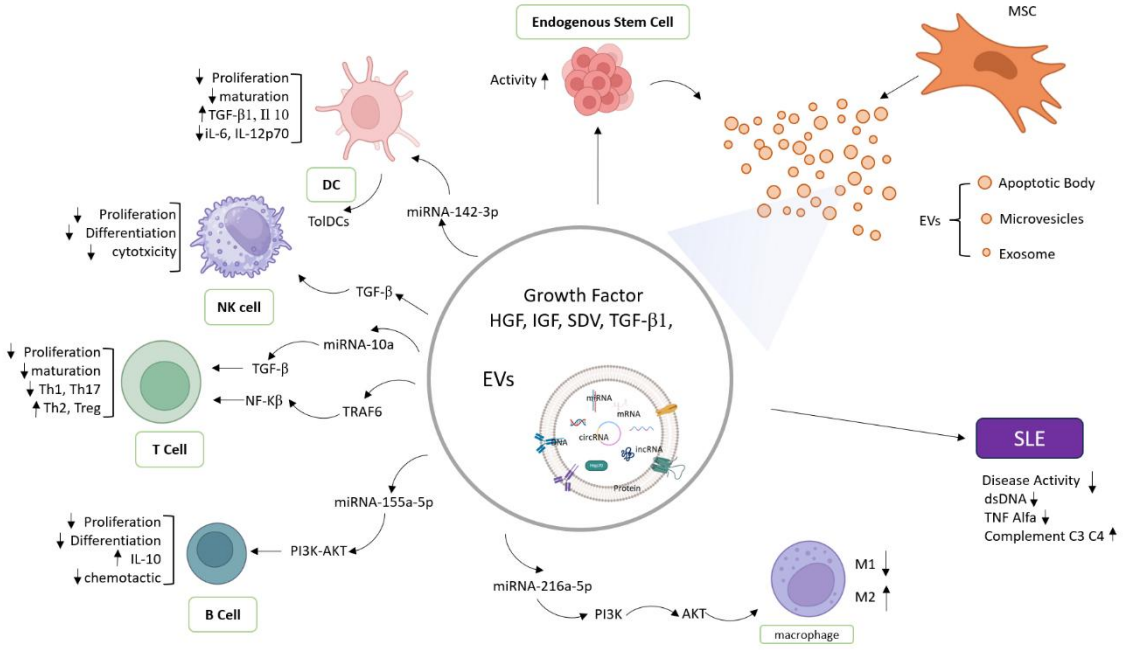

Figure 2. Mechanisms and composition of MSC-derived secretome in SLE and its immunological impact. The MSC-secretome comprises growth factors, cytokines, and extracellular vesicles (EVs), which play key roles in modulating immune responses in SLE. Growth factors such as transforming growth factor-beta 1 (TGF-β1), hepatocyte growth factor (HGF), stromal growth factor (SGF), and insulin-like growth factor (IGF) are crucial in immune regulation. EVs, lipid bilayer particles containing proteins, lipids, DNAs, non-coding RNAs, miRNAs, and mRNA, facilitate genetic information exchange and cell reprogramming via multiple signaling pathways. The MSC-secretome can inhibit B cell differentiation and proliferation through the Phosphatidylinositol 3-Kinase–Protein Kinase B (PI3K-AKT) pathway, reducing interleukin-10 (IL-10) production. T cells are modulated to decrease T helper (Th)17 and Th1 differentiation while enhancing Treg and Th2 function through the TGF-β/Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway. EVs inhibit dendritic cell (DC) growth and promote the development of tolerogenic DCs with reduced costimulatory markers. Additionally, EVs promote the M2 anti-inflammatory macrophage phenotype through the PI3K/AKT pathway. NK cell activity is suppressed through TGF-β signaling, limiting their pathogenic effects. MSC-secretome also stimulates the activation of endogenous stem cells capable of secretome production, thereby enhancing immunological tolerance in SLE.

The therapeutic application of MSC-derived secretome demonstrated notable effects on complement levels, particularly C3. In patients with lupus nephritis, complement C3 levels significantly improved by Day 43, with the secretome group showing superior recovery compared to controls. These findings are consistent with previous studies emphasizing the efficacy of MSC-based therapies in SLE management [12,23-25]. However, it is critical to acknowledge that factors such as medication adherence, psychological status, and environmental influences may also contribute to these improvements.

While complement C3 recovery is a marker of reduced disease activity, complement C4 ablation in SLE is often linked to B-cell depletion [26]. Similar to a 2020 meta-analysis, this study observed no significant changes in C4 levels on Days 22 and 43 [25]. The absence of significant C4 recovery may be explained by its heightened susceptibility to degradation, particularly during sample handling. C4 degradation occurs rapidly if blood samples remain at room temperature for 1–2 hours, likely due to activation of the classical C3 convertase pathway [26,27]. These findings underscore the importance of immediate sample processing to ensure accurate measurement of complement levels. The differential stability between complement C3 and C4 highlights the complexities in evaluating therapeutic responses. While C3 recovery reflects reduced disease

activity, the rapid degradation of C4 may obscure subtle therapeutic effects. Larger, more comprehensive studies with optimized sample handling protocols are warranted to validate these findings and elucidate the full scope of MSC-secretome's therapeutic potential in SLE management.

The study's findings demonstrated no significant decrease in TNF-α levels in the control group on Day 43 after the intervention compared to Day 22 and before the intervention. However, the control group exhibited a more substantial reduction in both IL-6 and TNF-α levels than the intervention group. This disparity was attributed to patients' baseline characteristics, including comorbidities and compromised nutritional status. Additionally, variations in medication usage, such as the influence of cyclosporine on IL-6 expression and IL-2 secretion, might have impacted baseline conditions [28,29]. Notably, the study's inability to exclude comorbid conditions represented a limitation, suggesting the need for future studies to implement tighter inclusion and exclusion criteria and ensure baseline matching to reduce bias [30]. Conversely, a significant reduction in IL-6 levels was observed 43 days after MSC secretome injection in the intervention group compared to the control group. This aligned with previous evidence indicating that MSCs and their conditioned media suppressed IL-6 production in macrophages [31]. Moreover, an in vitro study reported reduced TNF-α levels in bone marrow macrophages cultured with MSCs or their secretome [32]. These findings reinforced the potential of MSC secretome in modulating inflammatory responses.

Elevated IL-6 and TNF-α levels were strongly correlated with disease activity in SLE [33,34]. This study confirms a previous study indicating that mesenchymal stem cell secretome administration reduces these IL-6 and TNF-α levels [35]. The current study corroborated previous reports that MSC secretome administration reduced these cytokines, as demonstrated in SLE mouse models where MSCs lowered pro-inflammatory cytokines (IFN-γ, IL-2, TNF-α, IL-6, IL-12) while enhancing anti-inflammatory cytokines (IL-4 and IL-10) [24]. Furthermore, extracellular vesicles derived from MSCs were shown to mediate immunosuppressive functions by facilitating intercellular communication and delivering bioactive molecules [36-38]. MSCs were also found to induce anti-inflammatory phenotypes in macrophages, monocytes, and dendritic cells, inhibit T lymphocyte proliferation, and promote regulatory T cell differentiation [29,31]. The observed reduction in IL-6 and TNF-α levels highlighted the anti-inflammatory mechanisms of the UCMSC-derived secretome, particularly its ability to regulate pivotal inflammatory mediators in SLE. The suppression of these cytokines correlated with clinical improvement and provided evidence of direct immunomodulatory effects. By Day 43, the significant decline in pro-inflammatory cytokines aligned with preclinical studies showing that MSC secretome inhibited macrophage activation and reduced cytokine release [35,36]. This result indicates a direct immunoregulatory action of the secretome on inflammatory pathways.

The study observed a reduction in anti-dsDNA levels in both the secretome group and the control group. While the reduction in the secretome group did not achieve statistical significance, it demonstrated a more pronounced decrease compared to the control group. Supporting evidence from animal models of lupus nephritis further corroborates these findings. In such models, MSC-derived secretome administration resulted in a significant reduction in anti-dsDNA levels, comparable to those observed in negative controls (healthy mice) and markedly lower than in untreated positive controls [39]. This aligns with prior studies that have highlighted MSC therapy as an effective intervention for severe refractory SLE, demonstrating a consistent decrease in anti-dsDNA antibody concentrations post-therapy [38,40].

A key finding of the present study is the absence of severe adverse events associated with UCMSC-derived secretome administration. This exceptional safety profile underscores its potential for long-term application in SLE management. The significance of this finding is amplified in the context of existing SLE therapies, which often entail substantial risks such as heightened infection susceptibility, organ toxicity, and cardiovascular complications stemming from prolonged immunosuppression [41]. The observed safety advantages of the secretome approach highlight its promise as a therapeutic modality that mitigates these common complications. However, the study's primary limitation is its small sample size, a consequence of stringent inclusion and exclusion criteria. The limited participant pool may restrict the generalizability of the findings. To build on these initial results and enhance their external

validity, future research should prioritize multi-center studies involving larger and more diverse cohorts of SLE patients. Such studies would provide robust evidence to confirm the efficacy and safety of UCMSC-derived secretome therapy across varied patient populations.

Conclusion

The results of this study demonstrate that UCMSC-derived secretome is a safe and effective adjuvant therapy for SLE with moderate activity. The absence of severe adverse events following UCMSC-derived secretome administration highlights its safety profile. Efficacy was evident through significant reductions in disease activity, as reflected by the decrease in MEX-SLEDAI scores. Additionally, there were improvements in complement C3 levels, pro-inflammatory cytokines (IL-6 and TNF-α), and anti-dsDNA levels, although not statistically significant. These findings underscore the potential of UCMSC-derived secretome to modulate immune dysregulation in SLE and support its role in enhancing current therapeutic strategies. However, the relatively short duration of the study and the limited sample size warrant further investigation. Future studies with larger cohorts, extended follow-up periods, and more comprehensive outcome assessments are essential to confirm these results and fully establish the clinical efficacy and safety of UCMSC-derived secretome.

Ethics approval

The study received ethical approval from the Health Research Committee of Dr. Moewardi General Hospital, Surakarta, Indonesia (No. 1.157/IX/HREC/2022), and all participants provided informed consent.

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Competing interests

No potential conflicts of interest to disclose.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

Declaration of artificial intelligence use

This study utilized the artificial intelligence (AI) tool, Quillbot, for language refinement, which included improving grammar, sentence structure, and the overall readability of the manuscript. We confirm that all AI-assisted processes were critically reviewed by the authors to ensure the integrity and reliability of the results. The final decisions and interpretations presented in this article were made solely by the authors.

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