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Comparison of Short-Chain Fatty Acid (SCFA) and Interleukin-10 (IL-10) Levels in Patients with Systemic Lupus Erythematosus (SLE) Compared to Healthy Populations at RSUP Dr. Mohammad Hoesin Palembang

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ABSTRACT

Background. Systemic lupus erythematosus (SLE) is a prototypical autoimmune disease with various significant diseases, where SLE can affect the entire population in the world. This study aims to compare and analyze differences in the composition of the gut microbiota of SLE patients compared to healthy controls based on SCFA examination in Indonesia.
Methods. The type of research conducted in this research is an analytical observational study with a case-control design. The research was conducted at Dr. RSUP. Mohammad Hoesin Palembang from October 2020 to October 2021. The sample in this study was divided into two groups, namely, the case group, and the control group. The case group was all SLE patients who met the inclusion criteria, while the control group was a healthy population who did a medical check-up at Dr. RSUP. Mohammad Hoesin Palembang. SCFA examination was carried out using Gas Chromatography-Mass Spectrometry (GCMS) from fecal samples.
Results. The results showed that there were two groups of SCFA values, namely the normal group and the microbiota dysbiosis group where the SCFA value was low or less than 4. in the SLE and normal populations where there were 6 SLE subjects who had normal SCFA values or 37.5% and there were 10 SLE subjects who experienced microbiota dysbiosis. or 62.5%. In the healthy population group, all subjects had SCFA values that were included in the normal category, namely 16 subjects or 100%. Based on the severity of SLE using the SLEDAI MEX score, all SLE patients in the study were in the active or category *flare* where the SLEDAI MEX score was > 5.
Conclusion. Patients with microbiota dysbiosis tended to have an LES of 7,222 or 7 times greater than patients who did not have microbiota dysbiosis or had normal SCFA values.

1. Introduction

Systemic lupus erythematosus (SLE) is a complex chronic autoimmune inflammatory disease characterized by the presence of autoantibodies against cell nuclei and involves many organ systems in the body.[1] The clinical manifestations of each patient with SLE vary widely, ranging from skin, joints, and internal organ involvement that can be life-threatening.[2] LES is a prototypical autoimmune disease with a variety of significant diseases, in which the LES can be suffered by the entire population of the world, from various ethnic and racial prevalence found in most Asian and Hispanic populations.[3,4] SLE is a disease that mostly affects women of childbearing age, with the ratio of women to men being 9:1, with a mortality rate that increased to 2.3 – 3.3 times higher in 2004 compared to 2002.[5] In Indonesia, based on Data and Information Center (Pusdatin) of the Indonesian Ministry of Health in 2017, the incidence of new LES cases at RSUP Dr.

Moh Hoesin Palembang ranks 3 out of the top 8, and the incidence is considered quite high, which is an increase of 11.7% .[5,6]

The etiopathology of SLE is thought to involve a complex and multifactorial interaction between genetic variation and environmental factors[7]. Genetic factors are thought to play an important role in predisposing SLE disease, but in cases of SLE that occurs sporadically without identification of genetic factors, various environmental factors are thought to be involved in the etiopathology of this disease.[1,3,8] SLE is characterized by loss of immune system tolerance, resulting in hyperreactivity of T cells and T cells that increase the production of pathogenic autoantibodies and cause tissue damage.[8.9]

Based on the hygiene hypothesis, environmental factors that play a role in the autoimmune process, especially in the pathogenesis of SLE, are changes in the composition of the gut microbiota.[10,11] A research publication by Dwivedi M, et al in 2016 stated that changes in the composition of the gut microbiota could be associated with various autoimmune diseases such as systemic lupus erythematosus and was reinforced by a study in China by Wei F, et al in 2019 which showed that there were differences in the diversity of the microbiota. in SLE patients when compared with healthy controls. Changes in the composition and activity of the gut microbiota are called microbiota dysbiosis. The presence of intestinal microbiota dysbiosis causes hyperreactivity of the immune system and tissue damage.[11,12,13] Intestinal microbiota dysbiosis will increase inflammation, decrease anti-inflammatory agents, which cause B cell hyperreactivity so that it can increase the production of pro-inflammatory cytokines and increase the secretion of IgG anti-DNA antibodies, which results in tissue damage and increases the inflammatory process in SLE. .[14.15]

The composition of the gut microbiota can be detected by several methods, one of which can be seen from Short-Chain Fatty Acid (SCFA). SCFA is an alpha group in the form of a carboxylic acid which has 6 carbon chains, which is one of the end products of intestinal microbiota fermentation. Research by Venegas D, et al in 2019 suggested that SCFA is a microbiota metabolite that is closely related to the function of the gut microbiota for the recognition and regulation of the immune system and metabolism of cells and body tissues. Changes in the ratio of the gut microbiota will affect the levels of SCFA thereby increasing the inflammatory process, and can affect the severity of SLE and even trigger the appearance of SLE manifestations. SCFA was examined through a stool sample using a gas chromatography-mass spectrometry (GCMS) procedure.[14,15, 16]

Dysbiosis gut microbiota can reduce the activity of regulatory T. Regulatory T cells produce cytokines that can regulate the immune system by expressing IL-10, TGF-β and Foxp3+. IL-10 is an anti-inflammatory cytokine that plays a very important role in Treg cell differentiation and can suppress excessive immune response activity in SLE pathogenesis. The presence of dysbiosis of the gut microbiota can reduce the activity of regulatory T cells, thereby reducing the production of IL-10 cytokines, which causes hyperreactivity of the immune system.[17] Several theories and studies have suggested a role for the gut microbiota in SLE. However, there are no studies that compare and analyze differences in the composition of the gut microbiota of SLE patients compared to healthy controls based on the SCFA examination in Indonesia.

2. Methods

The type of research conducted in this research is an analytical observational study with a case-control design. The research was conducted at RSUP Dr. Mohammad Hoesin Palembang from October 2020 to October 2021. The subjects of the study were all SLE patients who were treated at the Internal Medicine Allergy Immunology section of RSMH Palembang. The sample in this study was divided into two groups, namely, the case group, and the control group. The case group was all SLE patients who met the inclusion criteria, while the control group was a healthy population who did a medical check-up at RSUP Dr. Mohammad Hoesin Palembang. The inclusion criteria for the case group were that all SLE patients had been diagnosed based on the 2017 Systemic Lupus International Collaborating Clinics (SLICC) criteria, aged over 18 years and willing to participate in the

study and signed informed consent. The inclusion criteria for the control group were a healthy population who did a medical check-up at Dr. RSUP. Mohammad Hoesin Palembang, with results that have been declared healthy and willing to participate in the study and sign the informed consent. A total of 32 research subjects participated in this study and were divided into two groups, 16 case groups and 16 control groups, where matched age and sex were in the case and control groups.

SCFA examination was carried out using Gas Chromatography-Mass Spectrometry (GCMS) from stool samples. The materials needed area. Standard Volatile Free Acid Mix, Supelco (Bellefonte, PA, USA), standard concentration of 10 mmol/kg, b. Internal Standard 2-2 Dimethyl Butyrate, Sigma-Aldrich (St.Louis, USA), c. Isopropanol and Methanol, Merck with Liquid Chromatography grade, d. Stool samples of 171 patients who had been given certain treatments, then placed at a temperature of -20°C. Standard preparation was a. The standard stock of Volatile Free Acid Mix in Isopropanol made serial dilution 2x with a concentration range of 0.00625 - 8.00 mmol/kg, b.100 L standard was diluted in a mixture of IPA: HCL 1.5 N (1:6) (IPA containing ISTD with a final concentration of 20 mg/mL), c. Homogenize by vortex for 1 minute. Sample preparation was 200 mg of faeces sample dissolved in 1 mL H2O (containing STD with a final concentration of 20 mg/ml, b. First sonification for 30 minutes to reduce faecal particles so that they are easily homogenized, c. Homogenize with vortex for 1 minute, dCentrifugation at 14,000 rpm for 10 minutes, e.100 L supernatant dissolved in 300 L IPA, f.100 L of faecal solution in IPA diluted 6x in a mixture of IPA:HCl 1.5 N (1:6), g. Homogenize with vortex for 1 minute, h.500 L supernatant transferred into amber GC vial and tightly closed. Instrumentation: GC conditions, Instruments: Agilent Technologies series 7890B. Column: Fused silica Capillary Column 30 mx 0.25 mm x 0.25 m film thickness. Column Temperature: 60 °C, Oven Temperature: 60-150 °C, Oven Temperature Rate: 10 °C/minute (for 20 minutes running). MS Condition, Instrument: Agilent Technologies 5977A MSD, Ion Source: Electron Ionization, Electron Multiplier Voltage: 1406 V, Acquisition type: SIM (Selected Ion Monitoring). The prepared sample was put into the GC Vial and then 1 L was injected into the GCMS system. The analysis results were read based on the retention time of the molecules from the GC separation and the peak area read from the MS. The sample concentration was read based on the calibration curve of the relationship between concentration and MS response. The results of the examination are SCFA values, with a normal range of 4-18 mg/mL.

The examination of IL-10 levels was carried out using the Enzyme-Linked Immunosorbent Assay (ELISA), where samples were taken from the subject's feces. The test sample was prepared by homogenization and then centrifuged at 10,000 rpm for 10 minutes, at 4°C, then the supernatant was taken and used for measuring IL-10 levels. The supernatant was put in a microplate that had been incubated with antibody anti-IL-10, then performed incubation at 37°C for 30 minutes, followed by addition of a secondary antibody conjugated HRP, Back conducted incubation and added chrome A and B then stop solution, then microplate inserted to a microplate ELISA reader at a wavelength of 450nm.

Data analysis was carried out with the help of SPSS 26 software. Univariate analysis was performed to see the distribution of levels presented in mean±SD. Then a bivariate analysis was performed to see the comparison of SCFA and IL10 levels between the case and control groups. The probability value is 5%, meaning that if the p-value is less than 0.05, then there are differences in the comparison of SCFA and/or IL10 levels between groups.

3. Results

Table 1. Characteristics of study subjects

Variable	N (%)	Mean ± SD	Median (range) *
Gender			
• Female	30 (93.7%)		
• Male	2 (6.3%)		
Age		29 ± 6 years	
Education			
• SMA / equivalent	14 (43.7%)		
• College	18 (56.3%)		
Occupation			
• Housewives	7 (21.9%)		
• Private	19 (59.4%)		
• Not working	4 (12.5%)		
• Students	2 (6.3%)		3.0 (0.0 – 10.0)
Score MEX SLEDAI			0.99 (0.12 – 25.88)
IL-10			
SCFA			
• Normal			
• Dysbiosis microbiota (SCFA <4 → low)	22 (68.8%) 10 (31.2%)		

*Shapiro Wilk P < 0.05

General characteristics of research subjects include gender, age, education, occupation, SLEDAI MEX score, and grade short-chain fatty acids or SCFA. There were 30 female subjects (03.8%) and 2 male subjects (6.3%). The average age of the subjects in the study was 29 years with a standard deviation of 6 years. Educational characteristics of the subjects in this study were SMA or high school as many as 14 subjects (43.7%) and universities as many as 18 subjects (56.3%). In this study, 7 subjects worked as housewives (21.9%). Meanwhile, for the private workgroup, not working, and students, 1 subject (3.1%), 4 subjects (12.5%) and 2 subjects (6.3%), respectively. The median value of the SLEDAI MEX score in the study was 3.0 with a range of 0.0 to 10.0. The median value of interleukin-10 or IL-10 in this study was 0.99 with a range of 0.12 to 25.88. Based on the SCFA scores, which are divided into 3 groups, namely low, normal and moderate with limits of less than four, four to eighteen, and more than eighteen, respectively, there are 22 subjects or 68.8% who fall into the normal SCFA category and 10 subjects or 31.2% with microbiota dysbiosis with an SCFA value of less than 4.

In this study, the SCFA and IL-10 values were examined and a comparison of the values of the laboratory characteristics is shown in table 4.2. The median value of SCFA in the SLE population is 3.0 with a range of 2.0 to 5.0 while the average value of SCFA in the normal population is 12.69 with a standard deviation of 2.89. The p-value or p-value of the comparison of SCFA values in the SLE and normal population has a statistically significant significance, namely p = 0.000 (p <0.05).

Table 2. Laboratory characteristics of research samples

Characteristics	**SLE** (n = 16)		**Normal Population** (n = 16)		p*
	Mean ± SD	Median (range)	Mean ± SD	Median (range)	
SCFA		3.0 (2.0 – 5.0)	12.69 ± 2.89		0.000*
IL- 10		1.75 (1.26 – 25.88)		0.15 (0.12 – 0.72)	0.000*

*Mann-Whitney test (p is significant if < 0.05)

The median value of IL-10 in the SLE population is 1.75 with a range of 1.26 to 25.88 while the median value of IL -10 in the normal population is 0.15 with a range of 0.12 to 0.72. The p-value of the comparison between the levels of IL-10 in the SLE population and the normal population had a statistically significant significance, namely p = 0.000 where p <0.05.

In this study, there were two groups of SCFA values, namely the normal group and the microbiota dysbiosis group where the SCFA value was low or less than 4. Table 3 presented data on the SCFA group in the SLE and normal population where 6 SLE subjects had a normal SCFA value or 37.5% and There were 10 SLE subjects with microbiota dysbiosis or 62.5%. In the healthy population group, all subjects had SCFA values that were included in the normal category, namely 16 subjects or 100%.

Table 3. Comparison of SCFA groups in SLE and healthy population

SCFA	**SLE (n = 16)**	**Healthy population (n = 16)**
Normal	6 (37.5%)	16 (100.0%)
Microbiota dysbiosis (SCFA < 4)	10 (62.5%)	0 (0.0%)

Based on the severity of SLE using the SLEDAI MEX score, all SLE patients in the study were in the active or category *flare* where the SLEDAI MEX score was > 5. Therefore, the assessment of IL-10 levels in the SLE population was reviewed based on the SLEDAI MEX scores in the SLE population. The research scores are 0, 8, 9, and 10 in table 4.

Table 4. IL-10 levels based on the severity of SLE

Degree of SLE (SLEDAI MEX score)	**N = 16**	**Median IL-10 (range)**
0	16	0.15 (0.12 – 0.72)
8	6	1.40 (1.26 – 4.86)
9	7	1.71 (1.26 – 25.88)
10	3	2.59 (1.41 – 6.07)

Subjects with a zero MEX SLEDAI score had a median IL-10 value of 0.15 with a range of 0.12 to 0.71. Subjects with a MEX SLEDAI score of 6 had a median IL-10 score of 6.41 while subjects with a MEX SLEDAI score had a median IL-10 of 1.40 with a range of 1.26 to 4.86. The median value of IL-10 in the group of subjects with MEX SLEDAI scores of 9 and 10 is 1.71 and 2.59, respectively, with a range of 1.26 to 25.88 in the MEX SLEDAI score group of 9 and a range of 1.41 to 6.07 for the subject group with a MEX SLEDAI score of 10.

In table 5 The median IL-10 data based on the SCFA group is presented, namely the normal SCFA group and the subject group with microbiota dysbiosis or SCFA less than 4. The median IL-10 value for the normal SCFA subject group is 0.16 with a range of 0.12 to 1.79. The median IL-10 value in the SCFA group was less than 4 or the group with microbiota dysbiosis was 3.73 with a range of 1.43 to 25.88.

Table 5. Characteristics of SCFA against IL-10

SCFA	n	Median IL-10 (range)
Normal (SCFA 4-18)	22	0.16 (0.12 – 1.79)
Microbiota dysbiosis (SCFA < 4)	10	3.73 (1.43 – 25.88)

Data analysis was performed to find out a measure of the association of SCFA with SLE in the study. There were four SLE subjects with microbiota dysbiosis or SCFA values less than 4. In the study, all normal populations or without SLE did not experience microbiota dysbiosis, namely 16 subjects. The total subjects who experienced microbiota dysbiosis were 10 subjects out of a total of 32 research subjects. Therefore, the odds ratio in the study was 7.222, which means that patients with microbiota dysbiosis tend to have SLE of 7.222 or 7 times greater than patients who do not have microbiota dysbiosis or have normal SCFA values.

Table 6. Association measures of SCFA and SLE

SCFA	SLE	Healthy Population	OR	p*
Normal	10	16		
Dysbiosis	6	0		
Total	16	16	7,222	

Because this study is a pilot study or an initial study, the cut-off value of IL-10 is sought using the ROC curve or ROC curve. From the ROC curve method, it was found that the ideal cut-off of IL-10 for the pilot study was 0.99 with 95% CI and sensitivity value = 1.0 while the value (1-specificity) = 0.

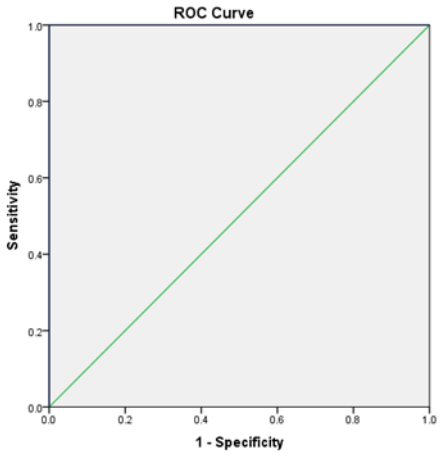

Table 7. Cut-off of IL-10

IL-10	SLE	population health	Total
Increases (≥0.99)	16	0	16
Decreases (<0.99)	0	16	16
Total	16	16	32

4. Discussion

Microbiota dysbiosis correlates with systemic lupus erythematosus where the total short-chain fatty acids present in patients with SLE can increase or decrease with a higher value of 18 or less than 4, respectively. These findings also illustrate that the potential association between microbiota dysbiosis and SLE disease affects endothelial activation in systemic lupus erythematosus by influencing the amount of SCFA in the body.[18] IL-10 correlates positively or unidirectionally with the activity of autoimmune diseases including systemic lupus erythematosus.[19-24] The higher the severity of the disease, the higher the increase or elevation of IL-10. This is following the results of studies that provide a similar picture or trend between the severity of IL-10 and the SLEDAI MEX score which represents the severity of lupus disease. Intestinal microbiota affects the production of SCFA in the body, under certain conditions such as autoimmunity, SCFA production will be disrupted and there is a dysfunction of regulatory T cell activation which is the cause of autoimmunity.[25-28] Due to the anti-inflammatory properties of IL-10, an increase in the number of SCFA will decrease IL-10 levels which will affect the outcome of a disease-grade activity. To determine the prediction of IL-10 threshold or cut-off in SLE patients and healthy populations, the ROC curve method was used as the basis for the search for cut-offs in the initial study or pilot study. From the cut-off that appears on the ROC curve, and IL-10 level of 0.99 is considered an ideal threshold value for determining IL-10 levels in SLE patients and healthy populations. By using a cut-off of IL-10 levels of 0.99 where IL10 levels greater than or equal to 0.99 are considered positive for an increase in IL-10, data obtained shows that all SLE populations fall into the category of increased IL-10 while all healthy populations fall into the low IL-10 group. or decreased where the level is less than IL-10. This finding is in line with studies in which IL-10 levels correlate with disease activity and severity of systemic lupus erythematosus.[29-35] Therefore, the finding that with an IL-10 level cut-off of 0.99 in which subjects with an IL-10 level greater than or equal to 0.99 were considered to have elevated IL-10 levels provides an ideal picture because all SLE subjects were admitted. in the IL-10 group increased and all subjects in the control group were included in the IL-10 group decreased or less than 0.99.

5. Conclusion

Patients with microbiota dysbiosis tended to have an LES of 7,222 or 7 times greater than patients who did not have microbiota dysbiosis or had normal SCFA values.

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