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Short Chain Fatty Acid (SCFA) Gut Biota and Interleukin 6 (IL-6) Related to the Severity of Systemic Lupus Erithematosus (SLE) at Dr. Mohammad Hoesin General Hospital Palembang

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ABSTRACT

Background. SLE is an autoimmune disease characterized by the presence of autoantibodies in the nucleus, deposition of immune complexes, and can attack various body tissues. Gut biota in this case short-chain fatty acids (SCFA) play a role in the inflammatory process in the SLE, while the levels of IL- 6 can also be associated with a response to inflammation which will be seen in the degree of SLE activity. This study aims to determine the correlation of SCFA Gut biota and IL - 6 with SLE degree activity. **Methods**: This study is a cross-sectional study with a correlation test design, conducted from November 2021-January 2022 at RSMH Palembang with the research subjects being all SLE patients seeking treatment at the Allergy-Immunology Division of RSMH Palembang with categories of mild and moderate-severe SLE activity degree. Examination of SCFA Gut biota using stool samples and serum IL-6 levels were associated with SLE activity degree. Statistical analysis of the correlation test with Spearman for numerical data not normally distributed, and continued with linear regression test to assess the multivariate analysis in this study. **Results:** The sample consisted of 32 patients, every 16 patients with mild and moderate-severe SLE activity degrees. The correlation between SCFA Gut biota with SLE activity degree was found to have a correlation coefficient of r=-0.777 with p=0.000. Correlation between IL-6 with SLE activity degree obtained a correlation coefficient of r=0.910 with p=0.000, while the correlation test between IL-6 and SCFA Gut biota obtained r=-0.633 with a value of p=0.000. Multivariate analysis found that 70.5% of SCFA Gut biota and IL-6 affected SLE activity degree. **Conclusion:** SCFA Gut biota and IL-6 had a significant correlation in statistical tests with the SLE activity degree.

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] SLE is a prototypical autoimmune disease with various significant diseases where SLE can affect all populations in the world, from various ethnicities and races, with the highest prevalence found in Asian and Hispanic populations.[3,4] SLE is a disease that mostly affects women of childbearing age, with a ratio of women compared to men is 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 SLE cases at Dr. Mohammad Hoesin General Hospital Palembang ranks 3 out of the top 8, and the incidence is also considered quite high, increasing by 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 to SLE disease, but in cases of SLE that occur sporadically without the 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 hyperactivity 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 biota 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 biota are called microbiota dysbiosis. The presence of intestinal microbiota dysbiosis causes hyperactivity of the immune system and tissue damage.[11,12,13] Dysbiosis of the Gut biota will increase inflammation, and reduce anti-inflammatory agents, which causes B cell hyperactivity so that it can increase the production of pro-inflammatory cytokines and increase the secretion of anti-DNA IgG antibodies, which results in tissue damage and increases the inflammatory process in SLE.[14,15] The composition of the Gut biota can be detected by several methods, one of which can be seen from the Short-Chain Fatty Acid (SCFA). SCFA is an aliphatic group in the form of a carboxylic acid that 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 are microbiota metabolites closely related to the function of the Gut biota for the recognition and regulation of the immune system and metabolism of cells and tissues of the body.

Changes in the ratio of the Gut biota 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 using a stool sample using the Gas Chromatography-Mass Spectrometry (GCMS) procedure.[14,15,16] Research conducted by Wallace et al. (2016) stated an increase in the level of interleukin-6 (IL-6) in the serum of SLE patients. Previously in 2007, Chun et al. had conducted a similar study, and the results showed a significant increase in serum IL-6 levels in active SLE patients. Interleukin-6 (IL-6) functions in both nonspecific and specific immunity, produced by mononuclear phagocytes, vascular endothelial cells, fibroblasts, and other cells in response to microbes and other cytokines. In SLE conditions, where there is a dysbiosis condition, there will be continuous inflammation due to the formation of autoantibodies against autoantigens in the gastrointestinal tract. It will cause immune complex deposits that will affect enterocyte cells in the digestive tract and will stimulate macrophage cells to produce IL-6 cytokines, which are associated with B lymphocytes and result in the formation of more autoantibodies against autoantigens. If this condition occurs continuously, it will have a clinical impact on SLE patients, namely increasing the degree of activity of the SLE disease itself.

This study aims to explore the relationship of short-chain fatty acids (SCFA) and IL-6 with the degree of SLE activity in Dr. Mohammad Hoesin General Hospital Palembang. To date, there has been no study linking the Gut biota SCFA and IL-6 with the degree of SLE activity.

2. Methods

The type of research in this study was an analytical observational study with a cross-sectional design to test the correlation between SCFA Gut biota and IL-6 with the degree of SLE activity. The research was conducted at Dr. Mohammad Hoesin General Hospital Palembang from November 2021 – to January 2022. The subjects studied were all SLE patients who were treated at the Internal Medicine Allergy Immunology section of RSMH Palembang. The inclusion criteria of the research of the subject were all SLE patients who 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. Meanwhile, subjects with SLE with other complications were excluded from this study. A total of 32 research subjects took part in this study, where the study was approved by the Medical and Health Research Ethics Commission of Dr. Mohammad Hoesin General Hospital Palembang (No. 131/kepkrsmh/2021).

SCFA examination was carried out by means of Gas Chromatography-Mass Spectrometry (GCMS) from a stool sample. The materials needed are a. 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.samples Stool from patients who have been given a certain treatment are then placed at a temperature of -20^{o}C. The standard preparation is a. The standard stock of Volatile Free Acid Mix in Isopropanol is serialized in 2x dilutions with a concentration range of 0.00625 - 8.00 mmol/kg, b.100 L standard diluted in a mixture of IPA: HCL 1.5 N (1:6) (IPA contains ISTD with a final concentration of 20 mg/mL), c. Homogenize by vortex for 1 minute. Sample preparation was 200 mg of fecal sample dissolved in 1 mL H2O (containing STD with a final concentration of 20 mg/ml, b. First sonification for 30 minutes to reduce fecal so that they are easily homogenized, c. Homogenize with a vortex for 1 minute, centrifuged at 14,000 rpm for 10 minutes, e.100 L supernatant dissolved in 300 L IPA, f.100 L of fecal 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 ^{o}C, Oven Temperature: 60-150 ^{o}C, Oven Temperature Rate: 10 ^{o}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 injected into the GCMS system. The analysis results were read based on the retention time of the molecule from the GC separation, and the peak area read from the MS. The sample concentration was read based on the calibration curve of the concentration relationship and the MS response. The results of the examination are SCFA values, with a normal range of 4-18 mg/mL.

The examination of IL-6 levels was carried out using the Enzyme-Linked Immunosorbent Assay (ELISA), where samples were taken from the subjects' blood. The test sample was prepared by homogenization process and then centrifuged at 5,000 rpm for 10 minutes at 25^{o}C. Then the supernatant was taken and used in measuring IL-6 levels. The supernatant was put into a microplate that had been incubated with anti-IL6 antibodies, then incubated at 37^{o}C for 30 minutes, followed by the addition of secondary antibody conjugated HRP, re-incubated, and added chrome A and B, then stop solution, then the microplate was inserted. To a microplate ELISA reader at a wavelength of 450 nm.

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 carried out to see the comparison of SCFA and IL6 levels in the research subjects. The probability value is 5%, meaning that if the p-value is obtained SLEs than 0.05, then there is a difference in the comparison of SCFA and/or IL6 levels.

3. Results

There were 20 subjects (62.5%) who experienced SLE duration of SLEs than or equal to 12 months, and 12 subjects (37.5%) who experienced SLE duration of more than 12 months. The median duration of subjects experiencing SLE duration was 5 months (with a range of 1 - 36 months). The median value of interleukin-6 in this study was 9.01 (with a range of 0.26 – 70.00). Based on the SCFA value of gut microbiota, subjects were divided into 3 groups: low (< 4), normal (4-18), and high (> 18), respectively, there were 6 subjects (18.8%) in the category of low Gut biota SCFA, 23 subjects (71.9%) in the normal Gut biota SCFA category, and 3 subjects (9.4%) in the high Gut biota SCFA category. The median SCFA value of Gut biota in this study was 7 (with a range of 2 - 29).

Table 1. Baseline characteristics

Variable	N (%)	Mean + SD	Median (range)*	P
Gender • Woman • Man	 31 (96.9) 1 (3.1)		1 (1-2)	0.000
Age (years) • ≤ 45 • > 45	 30 (93.7) 2 (6.3)		32 (19-55)	0.000
Education • junior high school • High School/Equivalent • College	 2 (6.3) 20 (62.5) 10 (31.3)	32.46 ±9.61		0.095
Job • Housewives • Private • Does not work • Student	 19 (59.4) 4 (12.5) 8 (25.0) 1 (3.1)			
SLE Activity Degree • ≤ 5 (mild) • > 5 (moderate-severe)	 16 (50.0) 16 (50.0)		5 (2-10)	0.000
SLE Duration (month) • ≤ 12 • > 12	 20 (62.5) 12 (37.5)		5 (1 – 36)	0.000
IL-6			9.01 (0.26 – 70.00)	0.000
Gut biota SCFA • Low (<4 pg/ml) • Normal (4-18 pg/ml) • High (>18 pg/ml)	 6 (18.8) 23 (71.9) 3 (9.4)		7 (2 – 29)	0.000

*Shapiro Wilk test p>0.05

Table 2 shows that SLE duration, Gut biota SCFA, and IL-6 were significantly correlated with SLE activity. SLE duration and Gut biota SCFA were negatively correlated with the degree of SLE activity. Meanwhile, IL-6 was positively correlated with the degree of SLE activity.

Table 2. Correlation test between variables and SLE Activity Degree

		N	R	p*
SLE Activity Degree	Age	32	-0.090	0.626
	Gender	32	-0.040	0.827
	SLE duration	32	-0.374	0.035*
	SCFA gut microbiota	32	-0.777	0.000*
	IL-6	32	0.910	0.000*

* Spearman correlation test, the p-value is significant if p <0.05

Table 3. Effect of the variable with degree activity SLE

Model	R	R *square*	*Adjusted* R *square*
	0.851	0.724	0.705

Based on statistical tests using multivariate linear regression analysis (table 3) it was found that the Gut biotaSCFA and IL-6 obtained an adjusted R square value of 0.705, which means that the Gut biotaSCFA and IL-6 can predict the degree of SLE activity by 70.5%.

4. Discussion

From the results of the study above, it can be observed that the SCFA value of Gut biota tends to decrease along with the increase in the degree of SLE activity. Subjects with moderate-to-severe SLE activity had low Gut biota SCFA values, and no subjects with moderate-severe SLE activity had Gut biota SCFA values in the range above normal. On the other hand, none of the subjects with mild SLE activity had low Gut biota SCFA values. This finding is in line with studies that suggest an association between decreased SCFA in SLE disease and an association between disease severity and disturbances in the gut microbiota. SLE patients tend to have a reduced amount of microbiota from the phylum Firmicutes and, conversely, over-representation of the microbiota from the phylum Bacteroidetes. This trend resulted in a shift in the microbiota ratio between the phylum Firmicutes and Bacteroidetes (F/B ratio). The F/B balance correlates with serum-free fatty acid (FFA) levels, where FFA levels are one of the predictors of cardiovascular complications in SLE patients. This finding also illustrates that the degree of SLE activity increases inversely with the SCFA values of the gut microbiota.[17-19] Other studies explain the role of the gastrointestinal microbiota, which is reflected in SCFA levels, in the digestive process, nutrient absorption, formation and defense of the gastrointestinal barrier and prevention of colonization by pathogenic bacteria, regulation of the intestinal epithelial regeneration cycle, to modulation of the activation and progression of the immune system.[20] Another study stated that the microbiota affects every segment of the gastrointestinal tract, starting from the intraluminal microbiota, the epithelial microbiota, the gastrointestinal mucosal barrier, and the lamina propria, which is rich in lymphocytes and plasma cells.[21] SCFAs produced by the gastrointestinal microbiota, particularly Bifidobacterium, lactobacillus, and other symbiotic bacteria, activate the FFAR2, FFAR3, or GPR109a receptors on enterocytes, resulting in the suppression of the inflammatory response through inhibition of nuclear factor -light chain in the pathway the B cell histone deacetylase, facilitates the movement of Tregs and glucagon-like-1-peptide to further modulate intestinal homeostasis. In addition, SCFA also plays a role by inducing the production of tolerogenic dendritic cells (DC) by inducing CD4+ naive to Tregs, which in turn will balance the inflammatory response of TH17 and TH1 activity.[22]

There is an increase in IL-6 in patients with inflammatory conditions, particularly in the cardiovascular system, and that IL-6 is a more accurate marker than C-Reactive Protein.[23] Significant increase

in IL-6 in the SLE population and a strong association between IL-6 levels and SLE disease activity in a parallel direction or a positive correlation.[23] The effect of IL-6 on the SLE occurs in several stages. First, IL-6 plays a role by inducing the maturation of naive cells into plasma cells and differentiation of cytotoxic T cells through up-regulation of IL-2 and IL-2R. Furthermore, IL-6 plays a role in the balance between TH17 and Tregs.

The limitations of this study were that the Gut biota analysis was not carried out by real-time poly chain reaction, so the diversity of the Gut biota had not been seen directly, and no dietary recall on the subjects of this study where the Gut biota was strongly influenced by diet or eating habits of the patient.

5. Conclusion

There was a significant relationship with a strong correlation strength between SCFA Gut biota and IL-6 with SLE activity degree. Based on multivariate analysis, it was found that 70.5% of the SCFA Gut biota and IL-6 could affect SLE activity degree in this study.

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