

# Patients with Systemic Lupus Erythematosus with Anxiety or Depression: Clinical Characteristics, Food Intake, and Gut Microbiota Profile

*Meutia Gebrina*<sup>1</sup>, *Alvina Widhani*<sup>1,2,3\*</sup>, *Rudi Putranto*<sup>1,2</sup>, *Murdani Abdullah*<sup>1,2</sup>,  
*Ikhwan Rinaldi*<sup>1,2</sup>, *Samsuridjal Djauzi*<sup>1,2</sup>, *Fransiscus D. Suyatna*<sup>4</sup>,  
*Beti Ernawati Dewi*<sup>5</sup>, *Andi Yasmon*<sup>5</sup>, *Susan Rahayu*<sup>6</sup>, *Amalia Shabrina*<sup>7</sup>

<sup>1</sup>Department of Internal Medicine, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia.

<sup>2</sup>Department of Internal Medicine, Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

<sup>3</sup>Universitas Indonesia Hospital, Depok, Indonesia.

<sup>4</sup>Department of Pharmacology and Therapeutics, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

<sup>5</sup>Department of Microbiology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

<sup>6</sup>Molecular Biology Unit, Integrated Laboratory, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

<sup>7</sup>Asih Husada Hospital, Banjar, West Java, Indonesia.

## \*Corresponding Author:

*Alvina Widhani, MD., PhD. Division of Allergy and Clinical Immunology, Department of Internal Medicine, Faculty of Medicine, Universitas Indonesia - Cipto Mangunkusumo Hospital. Jl. Diponegoro no. 71, Jakarta 10430, Indonesia. Email: [alvina.widhani@gmail.com](mailto:alvina.widhani@gmail.com)*

## ABSTRACT

**Background:** Depression and anxiety are prevalent among patients with systemic lupus erythematosus (SLE), and gut microbiota may be a contributing factor. This study aimed to investigate the clinical characteristics, food intake, and gut microbiota profiles of SLE patients with anxiety or depression. **Methods:** An analysis of secondary data was conducted. The primary study was conducted at Cipto Mangunkusumo Hospital, Jakarta, Indonesia, in 2017–2018. The inclusion criteria were: a diagnosis of SLE, age 18–60 years, and gastrointestinal symptoms. The data collected included clinical data, food intake, anxiety and depression scores, SLE disease activity, and stool samples. Sequencing of the 16S rRNA gene was performed to profile the gut microbiota using DNA extracted from the stool samples. **Results:** After excluding those with incomplete data, 41 patients were analyzed. Among the subjects, 53.66% and 14.63% had anxiety and depression, respectively. SLE patients with anxiety were significantly more likely to harbor *Bacteroides* compared to those without anxiety (33.45% vs. 9.78%;  $p=0.02$ ) and had lower levels of complement C3 (78.72 vs. 100.85 mg/dL;  $p=0.03$ ). SLE patients with anxiety or depression had significantly lower fat intake compared to those without these conditions (38.78 vs. 48.43 g/day;  $p=0.04$ , and 31.48 vs. 45.27 g/day;  $p=0.04$ ). A significant correlation was observed between the proportion of *Bacteroides* and SLE disease activity ( $p=0.02$ ). **Conclusion:** SLE patients with anxiety showed a significantly higher proportion of *Bacteroides* and a lower C3 level compared to those without anxiety. Fat intake was significantly lower among SLE patients with anxiety or depression compared to those without either condition.

**Keywords:** anxiety, depression, gut microbiota, systemic lupus erythematosus.

## INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease with unpredictable progression between flares and remission.<sup>1</sup> This characteristic, along with other biological aspects of SLE and related psychological, social, and environmental factors, can contribute to psychosomatic problems. Studies have shown depression and anxiety to be significantly more prevalent in patients with SLE than in healthy controls (HCs).<sup>2</sup> A meta-analysis by Zhang et al.<sup>3</sup> showed that the prevalences of depression and anxiety in patients with SLE are 30% and 40%, respectively. Some factors among patients with SLE are related to depression and anxiety; these include neurological problems, disability and pain, certain blood autoantibody and cytokine levels, socioeconomic status, and psychological resilience.<sup>2</sup> Gut microbiota may also contribute to such psychosomatic problems among patients with SLE. Yao et al.<sup>4</sup> reported that the composition of gut microbiota among patients with SLE with depression differs significantly from that among patients with SLE without depression and healthy subjects. Patients with SLE with depression show decreased ratios of *Firmicutes* to *Bacteroidetes* and species richness. A meta-analysis found a decreased gut microbiota diversity index among patients with SLE compared with HCs.<sup>5</sup> These findings suggest that gut microbiota play a role in SLE development and disease progression.<sup>6</sup>

The association between gut microbiota and psychiatric conditions is bidirectional: depression can alter gut microbiota and vice versa. The gut–brain axis links the gut and cognitive and emotional centers in the brain, mediated by the nervous, enteroendocrine, neuroendocrine, and immune systems.<sup>7</sup> Supplementation with probiotics can improve anxiety, depression, and stress.<sup>8</sup>

Gut microbiome dysregulation causes a pro-inflammatory response that can influence immune dysregulation among patients with SLE and affect the gut–brain axis.<sup>4</sup> Intestinal dysbiosis in SLE is related to the decreased production of short-chain fatty acids (SCFAs), which are gut microbiota metabolites that modulate intestinal epithelial cells and inhibit inflammatory response.<sup>9</sup> An impaired intestinal

barrier causes translocation of gut microbiota into the systemic circulation, which activates autoreactive B and T cells by molecular mimicry and affects the Treg/Th17 balance. The translocated bacteria also affect the expression of the interferon signature by plasmacytoid dendritic cells.<sup>9-11</sup> Interferon alpha might further contribute to the occurrence of depression by reducing the serotonin utilization rate.<sup>12</sup>

Literature on gut microbiota profiles among patients with SLE with anxiety or depression is limited. Given this background, the present study aimed to determine the clinical characteristics, food intake, and gut microbiota profiles of patients with SLE with gastrointestinal symptoms accompanied by anxiety or depression.

## METHODS

### Study Design and Participants

This cross-sectional study was conducted by analyzing secondary data from our previously published randomized, double-blind, placebo-controlled trial.<sup>13</sup> Subjects with incomplete data for anxiety and depression assessment were excluded.

The primary study was conducted at the outpatient clinic of Cipto Mangunkusumo Hospital, Jakarta, Indonesia, in 2017–2018. The participants of the primary study were recruited consecutively. The inclusion criteria for the primary study were patients with SLE under the American College of Rheumatology 1997 criteria, age 18–60 years, and the presence of gastrointestinal symptoms (e.g., abdominal pain, bloating, diarrhea, constipation). The exclusion criteria were: (1) pregnant or breastfeeding; (2) acute infection; (3) current antibiotic treatment; (4) had consumed yogurt or taken probiotic supplementation in the 3 weeks before recruitment; or (5) were taking corticosteroids (more than 20 mg prednisone or equivalent per day). There were 46 total participants in the primary study. Data, including demographic data, medication history, SLE disease activity scores, and Hospital Anxiety and Depression Scale (HADS) scores, were obtained as the baseline point from each participant, before treatment was initiated.<sup>13</sup>

### Ethics

This study was approved by the Research Ethics Committee of the Faculty of Medicine, University of Indonesia (registration ID: 804/UN2.F1/ETIK/2017). Written informed consent was obtained from all participants.

### Sample Size

We calculated the sample size using hypothesis testing formula for the correlation between gut microbiota and anxiety or depression. Minimum sample size was 30 subjects.

### Study Procedures

Demographic data, medication information, baseline SLE disease activity scores (Systemic Lupus Erythematosus Disease Activity Index 2000, SLEDAI-2K), and HADS scores were collected from the primary study database. Subjects with missing or incomplete data were excluded.

### Outcome Measures

Symptoms of anxiety and depression were assessed using the HADS questionnaire. The HADS is a self-reporting measure composed of 14 items: seven that measure anxiety and seven that measure depression. Anxiety and depression were defined as a HADS score  $\geq 8$  for the related domain. Data on SLE disease activity levels were assessed using the score on the Mexican version of the Systemic Lupus Erythematosus Disease Activity Index. The corticosteroid dose was calculated as the total dose per day equivalent to prednisone.

Dietary assessment was performed by a certified nutritionist using a 1-day food recall and a food frequency questionnaire. In addition to total calories and each nutrient component, the Dietary Inflammatory Index (DII) was also calculated using the formula from Shivappa et al.<sup>14</sup> to obtain the overall DII score. A more positive DII score indicates a pro-inflammatory diet.<sup>14,15</sup>

### Stool Sample Process

The patients were provided with stool nucleic acid collection tubes. After collection, stool samples were stored at  $-80^{\circ}\text{C}$ . DNA extraction was carried out after all samples had been collected. Details on the DNA extraction, polymerase chain reaction process, sequencing of 16S rRNA, and microbiome analysis are

described in our previous publication.<sup>13</sup> DNA extraction was performed using QIAamp DNA stool mini kits (QIAGEN, Hilden, Germany) according to the manufacturer's guidelines. DNA concentration and purity were measured with a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and a Qubit Fluorometer (Invitrogen Life Technologies, Carlsbad, CA, USA). The resulting amplicons were purified, quantified, pooled, and sequenced using MiSeq Reagent Kits (Illumina, San Diego, CA, USA), following the manufacturer's instructions.

### Statistical Analysis

Other statistical analyses were performed using IBM SPSS Statistics (version 20.0.0; IBM Corp., Armonk, NY, USA). Data were expressed as means  $\pm$  standard deviations (for normally distributed data) and medians (interquartile range for data with skewed distributions). Normality tests were conducted using the Shapiro–Wilk test. Comparisons of numeric data between the two groups were analyzed using independent *t*-tests if the data were normally distributed. Otherwise, the comparisons were analyzed using the non-parametric Mann–Whitney *U* test. Correlations were analyzed using Pearson's correlation when normally distributed, or Spearman's correlation when not normally distributed. P-values  $< 0.05$  were considered statistically significant.

## RESULTS

All subjects in this study were female ( $n=41$ ), with a median (range) age of 33 (19–47) years. Five subjects from the primary study were excluded because of incomplete data. The median (range) body mass index (BMI), SLEDAI-2K score, and steroid dose were 21.60 (12.99–41.08)  $\text{kg}/\text{m}^2$ , 12 (4–32), and 5 (0–20) mg equivalent to prednisone per day, respectively. The proportions of SLE organ involvement were musculoskeletal (90.24%), mucocutaneous (70.73%), nephritis (41.46%), hematology (17.07%), and neuropsychiatric (9.76%). Steroid-sparing agents were hydroxychloroquine (12.20%), mycophenolate mofetil (73.17%), azathioprine (14.63%), and

cyclosporine (4.88%).

The mean ( $\pm$  standard deviation) HADS-A and HADS-D scores were 7.88 (2.82) and 4.83 (2.85), respectively. The proportions of patients with anxiety and depression in the present study were 53.66% and 14.63%, respectively: five subjects (12.20%) had both anxiety and depression, 17 (41.46%) had only anxiety, one (2.44%) had only depression, and 18 (43.90%) did not have anxiety or depression.

The 16s rRNA gene microbiome data from 41 fecal samples were included in the data analysis. The rarefaction curves for the microbiome samples showed a trend toward plateauing, indicating that sequencing depths were sufficient to represent most microbial species (**Figure 1a**). Figure 1b and 1c show the results of the principal coordinate analysis comparing patients with SLE with and without anxiety (**Figure 1b**) and patients with SLE with and without depression (**Figure 1c**).

A comparison of the demographic and clinical characteristics and gut microbiota profiles between patients with SLE with and without anxiety is shown in Table 1. The median disease duration and SLEDAI-2K score were higher in the anxiety group than in the non-anxiety group, although this difference was not significant. Complement factor C3 levels were significantly lower in SLE patients with anxiety than in those without anxiety. Food intake, particularly fat intake ( $p=0.04$ ), was lower among SLE patients with anxiety than among those without anxiety. The proportion of *Bacteroides* was significantly higher among those with SLE and anxiety than among those without anxiety, whereas the proportion of Proteobacteria was significantly lower. The diversity indices were lower in SLE patients with anxiety than in those without anxiety, but these differences were not significant.

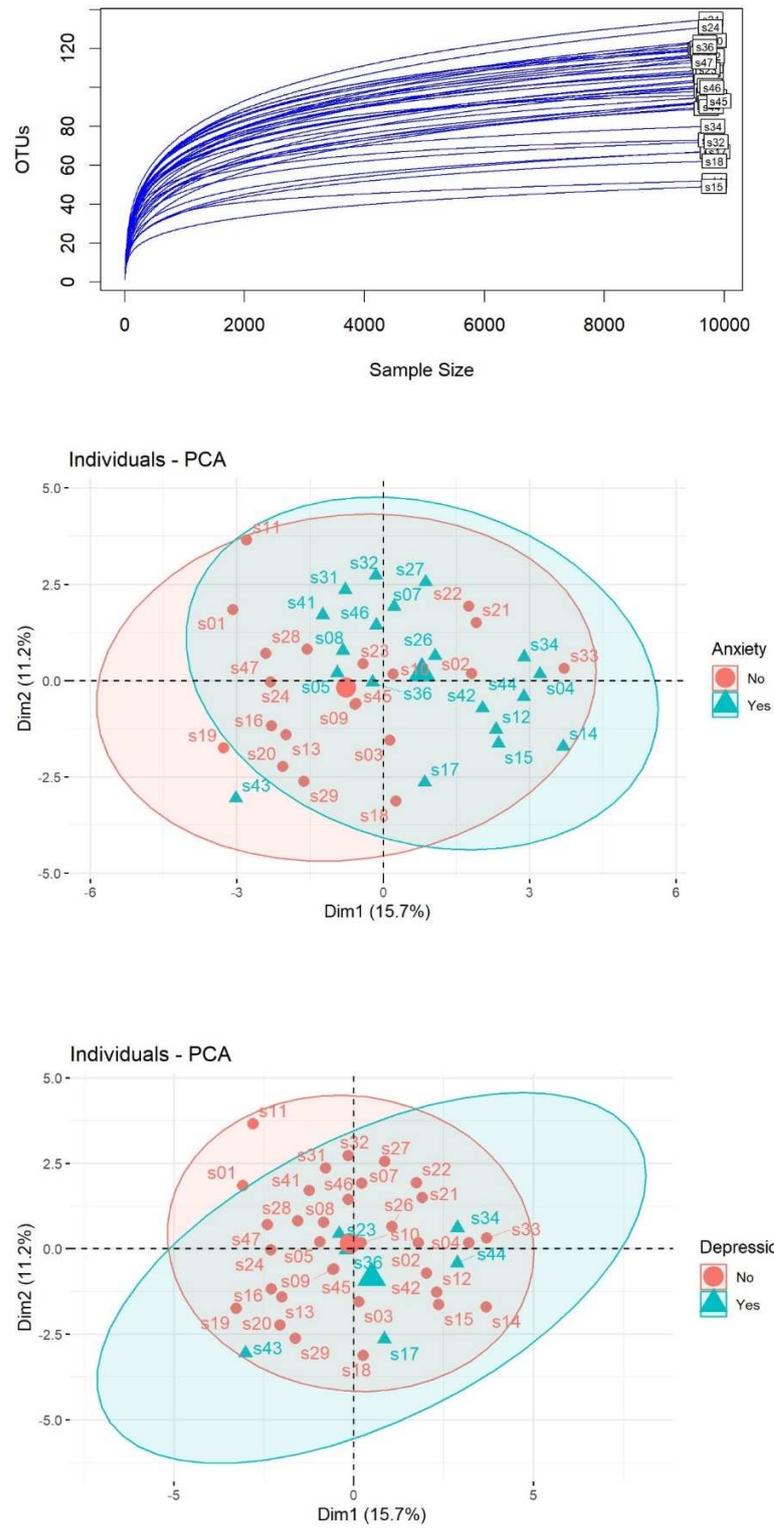
A comparison of patient characteristics and gut microbiota profiles between SLE patients with and without depression is also shown in **Table 1**. The use of proton pump inhibitors was higher among SLE patients with depression (83.3%) than among those without depression (42.9%). Food intake, particularly fat intake ( $p=0.04$ ), was lower, while the DII was higher

in SLE patients with depression than in those without depression. Among SLE patients with depression, the proportions of Bacteroidetes and Bacteroides were higher, whereas the proportions of Firmicutes and Proteobacteria, as well as the diversity indices, were lower than in those without depression; however, these differences were not significant.

Samples were grouped based on operational taxonomic units (OTUs) of the microbiome data and clinical variables, and then visualized by biplot principal component analysis (PCA) (**Figure 2**). This was done with the most abundant 25 OTUs. A list of OTUs can be seen in **Supplementary Table 1 (attached)**.

**Figure 2** shows that DII was positively related to *Escherichia fergusonii* (OTU 012) and negatively related to *Dialister succinatiphilus* (OTU 022), whereas BMI was positively related to *Enterobacteriaceae* (OTU 024). *Bacteroides* (OTU 002) and *Bacteroides dorei* (OTU 009) were related to HADS-A scores. This finding was also supported by analysis using the Mann–Whitney U test, which showed that the proportion of *Bacteroides* was significantly higher among SLE patients with anxiety than among those without anxiety (33.45% vs. 9.78%, respectively;  $p=0.02$ ) (**Table 1**). A significant correlation was also found between the proportion of *Bacteroides* and HADS-A scores ( $r=0.35$ ,  $p=0.03$ ) by Spearman's correlation. **Figure 2** also shows that HADS-D scores were related to *Bacteroides massiliensis* (OTU 020).

As shown in **Figure 2**, SLEDAI-2K scores were related to *Bacteroides stercoris* (OTU 007). This was supported by correlation analysis using Spearman's correlation, which revealed that the proportion of *Bacteroides* was significantly correlated with SLE disease activity ( $r=0.36$ ,  $p=0.02$ ). Diversity indices (Chao1, Shannon, and Richness index) also showed a significant correlation with SLE disease activity ( $r=-0.34$ ,  $p=0.03$ ;  $r=-0.38$ ,  $p=0.01$ ; and  $r=-0.33$ ,  $p=0.03$ , respectively). **Figure 2** also shows that C4 was related to *Sutterella stercoricanis* (OTU 015) and negatively related to *B. stercoris* (OTU 007), while anti-dsDNA was negatively related to *Clostridium XIVa* (OTU 025).

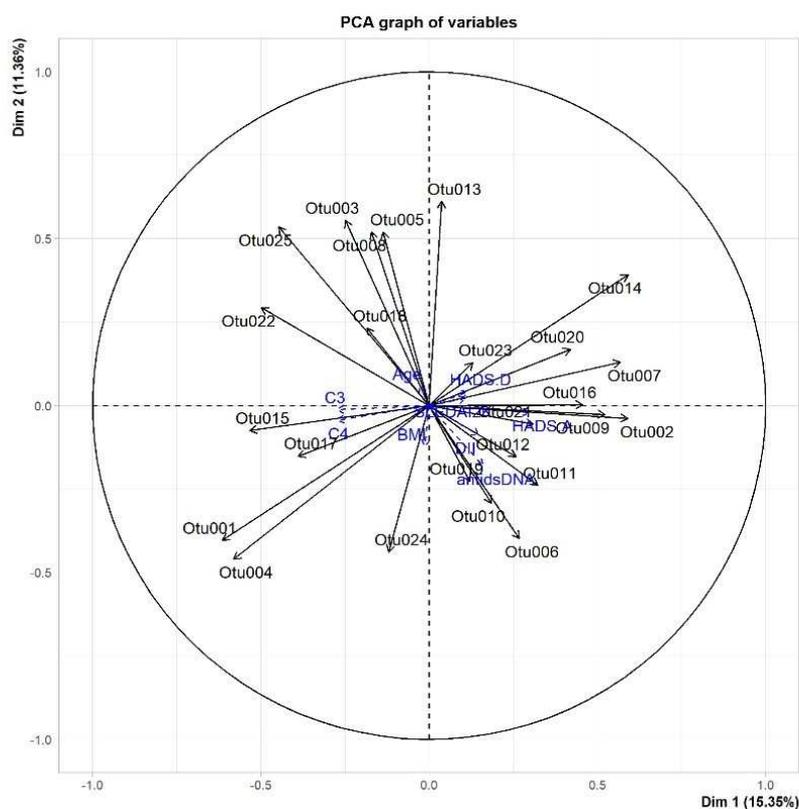


**Figure 1.** Rarefaction curve (a) and Principal coordinate analysis (PCoA) comparing SLE patients with and without anxiety (b) and with and without depression (c).

**Table 1. Clinical characteristics, food intake, and gut microbiota profiles of SLE patients with anxiety or depression**

Characteristics	Anxiety		p	Depression		p
	Yes (n=22)	No (n=19)		Yes (n=6)	No (n=35)	
Age (years), mean (SD)/median (min-max)	30.14 (7.98)	33.21 (8.87)	0.25	30 (24-47)	33 (19-45)	0.64
Disease duration (years), median (min-max)	3 (0.5-9)	2.5 (0.75-19)	0.48	2 (0.58-7)	3 (0.5-19)	0.19
BMI (kg/m <sup>2</sup> ), mean (SD)/median (min-max)	21.43 (12.99-28.35)	21.60 (17.36-41.10)	0.46	23.64 (9.58)	22.16 (3.92)	0.72
SLEDAI-2k, median (min-max)	14 (4-32)	10 (6-22)	0.41	12 (6-18)	12 (4-32)	0.88
Steroid use (mg/day equivalent to prednisone), median (min-max)	5 (0-20)	5 (0-20)	0.54	5.5 (5-10)	5 (0-20)	0.37
Concomitant drug use						
PPI, n (%)	10 (45.45%)	10 (52.63%)	0.65	5 (83.33%)	15 (42.86%)	0.09
Food intake						
Calories (kcal/day), mean (SD)	1185.45 (425.68)	1447.32 (384.57)	0.05	1048.28 (452.86)	1351.12 (408.42)	0.12
Carbohydrate (g/day), mean (SD)	169.20 (65.21)	204.36 (59.31)	0.08	158.44 (81.43)	190.14 (61.10)	0.27
Fat (g/day), mean (SD)	38.78 (14.05)	48.43 (15.51)	<b>0.04</b>	31.48 (9.09)	45.27 (15.38)	<b>0.04</b>
Protein (g/day), mean (SD)	42.58 (21.76)	53.12 (21.08)	0.12	33.60 (15.88)	49.84 (22.01)	0.09
Fiber (g/day), median (min-max)	8.38 (1.75-38.80)	10.49 (2.59-37.01)	0.25	6.48 (1.75-10.78)	9.85 (2.59-38.80)	0.05
PUFA (g/day), mean (SD)/median (min-max)	6.38 (3.93)	8.93 (5.61)	0.10	4.50 (0.99-7.24)	6.40 (1.80-25.21)	0.06
Diet inflammatory index (DII), median (min-max)	3.32 (-0.48-4.18)	3.41 (-0.03-4.07)	0.37	3.80 (2.87-4.12)	3.26 (-0.48-4.18)	0.07
Gut microbiota						
<i>Bacteroidetes</i> proportion (%), mean (SD)	53.21 (12.59)	51.56 (13.52)	0.69	53.07 (8.08)	52.34 (13.63)	0.90
<i>Firmicutes</i> proportion (%), mean (SD)	33.68 (15.29)	32.21 (12.50)	0.74	30.32 (11.88)	33.46 (14.34)	0.62
<i>Actinobacteria</i> proportion (%), median (min-max)	0.50 (0.03-9.1)	(0.05-2.30)	0.23	0.76 (0.48-2.31)	0.53 (0.03-9.10)	0.24
<i>Verrucomicrobia</i> proportion (%), median (min-max)	0 (0-2.67)	(0-1.23)	0.54	0 (0-0.02)	0 (0-2.67)	0.56
<i>Proteobacteria</i> proportion (%), median (min-max)	6.18 (1.83-40.50)	(4.39-37.30)	<b>0.02</b>	7.16 (1.83-40.50)	7.83 (2.03-37.30)	0.93
<i>Fusobacteria</i> proportion (%), median (min-max)	0.04 (0-26.7)	0.06 (0-3.21)	0.92	0.15 (0-0.71)	0.04 (0-26.70)	0.35
<i>Firmicutes/Bacteroidetes</i> ratio, median (min-max)	0.58 (0.18-3.60)	0.55 (0.22-1.60)	0.86	0.58 (0.21-1.03)	0.55 (0.18-3.6)	0.82
Genus <i>Bacteroides</i> proportion, median (min-max)/mean (SD)	33.45 (1.15-66.90)	9.78 (0.48-66)	<b>0.02</b>	28.32 (29.28)	26.44 (20.89)	0.85
Diversity index						
Richness index, mean (SD)	846.45 (150.70)	873.26 (112.35)	0.53	783 (187.32)	871.88 (120.72)	0.13
Shannon index, mean (SD)	4.36 (0.44)	4.48 (0.39)	0.36	4.25 (0.50)	4.45 (0.40)	0.30
Chao1 index, mean (SD)	852.45 (151.71)	879.77 (113.20)	0.52	787.55 (187.92)	878.41 (121.60)	0.13
Immunological parameter						
C3, mg/dL, mean (SD)	78.72 (32.08)	100.85 (29.22)	<b>0.03</b>	77.07 (55.12)	91.02 (27.54)	0.57
C4, mg/dL, mean (SD)	16.17 (6.74)	19.60 (10.50)	0.21	21.05 (15.77)	17.20 (7.15)	0.58
Anti-dsDNA, IU/mL, mean (SD)/median (min-max)	431.64 (364.84)	241.69 (282.55)	0.07	247.60 (2.20-1140.80)	262.80 (1-1030.90)	0.80

SD, standard deviation; BMI, body mass index; SLEDAI-2k systemic lupus erythematosus disease activity index 2000; PPI, proton pump inhibitor; PUFA, polyunsaturated fatty acid; anti-dsDNA, anti-double-stranded deoxyribonucleic acid.



**Figure 2.** Principal component analysis (PCA) of operational taxonomic unit (OTU) and clinical variables.

## DISCUSSION

The prevalence rates of anxiety and depression in patients with SLE vary.<sup>16</sup> The proportion of patients with SLE with anxiety identified using the HADS questionnaire in the present study was 53.66%. This result was similar to a study conducted by Kwan et al., who also used HADS scores and reported an anxiety prevalence of 50%.<sup>16</sup> However, the present finding was higher than that in a study by de Almeida Macêdo et al. (38.9%)<sup>17</sup> and a meta-analysis by Moustafa et al. (38.3%).<sup>18</sup> The proportion of patients with SLE with depression identified using the HADS questionnaire in the present study was 14.63%, which was lower than that in the study by Kwan et al. (29%)<sup>16</sup> and the meta-analysis by Moustafa et al., who reported that the pooled prevalence of depression was 24.4%.<sup>18</sup> Anxiety and depression can cause significant morbidity among patients with SLE, and some inflammatory cytokines are associated with anxiety disorders.<sup>18</sup>

In the present study, the median SLE disease activity score was higher in patients with anxiety

than in those without anxiety. High disease activity might be a physical stressor for patients with SLE, which in turn can cause anxiety.<sup>19</sup> We found that C3 levels were significantly lower in SLE patients with anxiety than in those without anxiety. This finding was similar to that by Shatri et al.,<sup>20</sup> who reported a significant negative correlation between anxiety symptoms and C3 levels.

We also observed lower food intake among patients with depression. Moreover, the median DII was higher among patients with SLE with depression than in those without depression. A higher DII means a more pro-inflammatory diet intake.<sup>15</sup> From the biplot PCA, we found that DII was positively related to *E. fergusonii* (OTU 012) and negatively related to *D. succinatiphilus* (OTU 022). *E. fergusonii* is an emerging opportunistic pathogen with high phenotypic and genotypic similarity to *Escherichia coli*.<sup>21</sup> *D. succinatiphilus* plays a role in maintaining an appropriate immune response by decarboxylating succinate to

propionate, which binds to the GPR-43 expressed in lymphocytes.<sup>22</sup>

*Bacteroides*, the main genus in the phylum *Bacteroidetes*, is a highly virulent pathogenic microbe that triggers inflammatory reactions in the intestine.<sup>23</sup> *Bacteroides fragilis* is a facultative, gram-negative, anaerobic bacterium that comprises a large proportion of the gut microbiota. It secretes various neurotoxins, including the highly pro-inflammatory *B. fragilis* lipopolysaccharide (BF-LPS).<sup>24</sup> BF-LPS can trigger the production of nuclear factor kappa-light-chain-enhancer of activated B cells, which causes the transcription of a group of pro-inflammatory microRNAs.<sup>25</sup>

In the present study, the proportion of *Bacteroides* was significantly higher among those with than without anxiety, and this was correlated with anxiety scores. Another study also found that the proportions of *Bacteroides* were higher among mouse models of lupus and patients with SLE compared with HCs,<sup>26,27</sup> and increased in proportion among patients with generalized anxiety disorder.<sup>28</sup> Furthermore, a study among patients with primary Sjögren's syndrome showed that *Bacteroides* was higher among an anxiety group compared with a non-anxiety group.<sup>29</sup> From the PCA in the present study, we found that HADS-A scores were related to *Bacteroides* (OTU 002) and *B. dorei* (OTU 009), whereas HADS-D scores were related to *B. massiliensis* (OTU 020). Jacobs et al.<sup>30</sup> reported that *B. dorei* was increased among patients with irritable bowel syndrome, while Ritz et al.<sup>31</sup> reported that *B. massiliensis* was increased in patients with social anxiety disorder compared with HCs.

In the present study, we also found a lower proportion of *Firmicutes* and a lower *Firmicutes/Bacteroidetes* ratio among SLE patients with depression. *Firmicutes* are the main gut microbiota that produce SCFAs<sup>23</sup> as well as several neurotransmitters.<sup>32</sup> A lower proportion of *Firmicutes* may lead to inflammation and the deficiency of many neurotransmitters, thereby resulting in depression. Our findings are consistent with those by Jiang et al.,<sup>33</sup> who also described a decrease in *Firmicutes* levels and an increase in *Bacteroidetes* levels in patients with

depression.

The proportion of *Proteobacteria* was significantly lower among those with than among those without anxiety. Nguyen et al.<sup>34</sup> reported that *Proteobacteria* were decreased among patients with schizophrenia compared with HCs. A similar result was found in the salivary microbiome of a patient with schizophrenia and patients with primary Sjögren's syndrome.<sup>35</sup>

In the present study, we also found lower microbial diversity in SLE patients with anxiety or depression compared to those without these conditions. A loss of microbial diversity is the most constant finding in gut dysbiosis and may affect the function of the microbial community. High biodiversity helps maintain a stable ecosystem so that the gut maintains resilience to many insults.<sup>36</sup>

Inflammation causes the conversion of tryptophan, the precursor of serotonin, to kynurenine, which causes serotonin deficiency.<sup>12</sup> A deficiency of GABA and serotonin can also occur because GABA- and serotonin-producing microbes are suppressed by pathobionts.<sup>32</sup> GABA deficiency plays a role in the pathogenesis of anxiety,<sup>32</sup> whereas serotonin deficiency plays a role in depression.<sup>8</sup>

We showed that the proportion of *Bacteroides* had a significant positive correlation with SLE disease activity, whereas diversity indices had a significant negative correlation. A high proportion of *Bacteroides* has been shown to have an inverse correlation with the serum levels of C3.<sup>10</sup> From the PCA in the present study, we found that SLEDAI-2K scores were related to *B. stercoris* (OTU 007), whereas C4 levels were negatively related. *B. stercoris*, a biomarker of the microbiota's resistance to structural changes, is found in abundance among individuals in whom lifestyle interventions have only a minor impact.<sup>37</sup> C4 levels are also related to *S. stercoricanis* (OTU 015). However, the role of *Sutterella spp.* in human disease remains controversial. *Sutterella* are commensals that do not significantly contribute to disrupted epithelial homeostasis.<sup>38</sup> We also found that anti-dsDNA was negatively related to *Clostridium XIVa* (OTU 025), a butyrate-producing species that accounts for 60% of the mucin-adhered microbiota.<sup>39</sup>

In the present study, associations were found between the DII and gut microbiota, gut microbiota and SLE disease activity, and gut microbiota and anxiety or depression. The DII was associated with *E. fergusonii*, an emerging opportunistic pathogen, and inversely related to *D. succinatiphilus*, a bacterium that helps maintain an appropriate immune response. Diet plays a significant role in shaping the gut microbiota.<sup>40</sup> Diets high in inflammatory foods, such as red meats, processed foods, and refined carbohydrates, can promote the growth of pro-inflammatory gut bacteria, leading to increased systemic inflammation, which may contribute to higher disease activity and a greater prevalence of depression or anxiety in patients with SLE.<sup>41</sup> These findings emphasize the importance of a holistic and comprehensive approach to managing SLE. Personalized interventions targeting inflammation through diet and modulation of the gut microbiota may help improve both SLE disease activity and psychological well-being in patients with SLE, in addition to the usual treatments.

This study has some limitations. First, the sample size was small because we used secondary data from a previous study. We only assessed anxiety and depressive symptoms based on a questionnaire, not on interviews. Second, psychosomatic disorders have various influencing factors, including educational background and social and economic factors, which were not assessed in this study. Third, the dietary assessment was performed using a food recall, which may have introduced recall bias. Participants were asked to recall and report their food consumption, which could lead to inaccuracies because of selective recall, faulty memory, or social desirability bias. However, this potential bias was considered to be minimized because the assessment was conducted by a certified nutritionist who used interviews with clear guidance and food models, which provided visual aids to help the participants estimate portion sizes more accurately.

## CONCLUSION

In the present study, SLE patients with anxiety harbored a significantly higher proportion of *Bacteroides* and a significantly lower proportion

of *Proteobacteria* and C3 levels compared with those without anxiety. In addition, fat intake was significantly lower among SLE patients with anxiety or depression compared to those without these conditions.

## ACKNOWLEDGMENTS

We would like to thank Ade Rosanti for assisting with the food intake analysis, Nida Makiyah and Indri for assisting with the DNA extraction, and Zulfikar Achmad Tanjung and Redi Aditama for assisting with the bioinformatics analysis.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## CONSENT TO PARTICIPATE

Written informed consent was obtained from all participants.

## DATA AVAILABILITY STATEMENT

The metagenomics data for the microbiome sequencing have been uploaded to the Sequence Read Archive under accession number PRJNA761343.

## FUNDING

This publication was supported by a PUTI grant from the University of Indonesia NKB-4443/UN2.RST/HKP.05.00/2020.

## AUTHORSHIP

MG, AW, RP, MA, IR, SD, FDS, and BED contributed to the study conceptualization; MA, IR, and AS contributed to the methodology; MG, AW, and AS contributed to the investigations; MG, AY, SR, and AS contributed to the formal analysis; MG and AW contributed to writing the original draft; and RP, MA, IR, SD, FDS, BED, AY, SR, and AS contributed to editing the manuscript.

## REFERENCES

1. Tselios K, Gladman DD, Touma Z, et al. Disease course patterns in systemic lupus erythematosus. *Lupus*. 2019;28:114–22. doi: 10.1177/0961203318817132.
2. Figueiredo-Braga M, Cornaby C, Cortez A, et al.

- Depression and anxiety in systemic lupus erythematosus: the crosstalk between immunological, clinical, and psychosocial factors. *Medicine*. 2018;97(28):e11376. doi: 10.1097/MD.00000000000011376.
- Zhang L, Fu T, Yin R, et al. Prevalence of depression and anxiety in systemic lupus erythematosus: a systematic review and meta-analysis. *BMC Psychiatry*. 2017;17(1):70. doi: 10.1186/s12888-017-1234-1.
  - Yao H, Yang H, Wang Y, Xing Q, Yan L, Chai Y. Gut microbiome and fecal metabolic alteration in systemic lupus erythematosus patients with depression. *Front Cell Infect Microbiol*. 2022;12:1040211. doi: 10.3389/fcimb.2022.1040211.
  - Xiang S, Qu Y, Qian S, et al. Association between systemic lupus erythematosus and disruption of gut microbiota: a meta-analysis. *Lupus Sci Med*. 2022;9(1): e000599. doi: 10.1136/lupus-2021-000599.
  - de Oliveira GLV, Leite AZ, Higuchi BS, et al. Intestinal dysbiosis and probiotic applications in autoimmune diseases. *Immunology*. 2017;152(1):1-12. doi: 10.1111/imm.12765.
  - Rogers GB, Keating DJ, Young RL, et al. From gut dysbiosis to altered brain function and mental illness: mechanisms and pathways. *Mol Psychiatry*. 2016;21(6):738-48. doi: 10.1038/mp.2016.50.
  - Taylor AM, Holscher HD. A review of dietary and microbial connections to depression, anxiety, and stress. *Nutr Neurosci*. 2020;23(3):237-50. doi: 10.1080/1028415X.2018.1493808.
  - Chen Y, Lin J, Xiao L, et al. Gut microbiota in systemic lupus erythematosus: a fuse and a solution. *J Autoimmun*. 2022;132:102867. doi: 10.1016/j.jaut.2022.102867.
  - Yao K, Xie Y, Wang J, et al. Gut microbiota: a newly identified environmental factor in systemic lupus erythematosus. *Front Immunol*. 2023;14:1202850. doi: 10.3389/fimmu.2023.1202850.
  - Pan Q, Guo F, Huang Y, et al. Gut microbiota dysbiosis in systemic lupus erythematosus: novel insights into mechanisms and promising therapeutic strategies. *Front Immunol*. 2021;12:799788. doi: 10.3389/fimmu.2021.799788.
  - Jeon SW, Kim YK. Inflammation-induced depression: its pathophysiology and therapeutic implications. *J Neuroimmunol*. 2017;313:92-8. doi: 10.1016/j.jneuroim.2017.10.016.
  - Widhani A, Djauzi S, Suyatna FD, et al. Changes in gut microbiota and systemic inflammation after synbiotic supplementation in patients with systemic lupus erythematosus: a randomized, double-blind, placebo-controlled trial. *Cells*. 2022;11(21):3419. doi: 10.3390/cells11213419.
  - Ardini W, Bardosono S. Using the dietary inflammatory index for assessing inflammatory potential of traditional cuisine from four ethnic groups in Indonesia. *Journal of Food and Nutrition Research*. 2020;8(4):168-71. doi: 10.12691/jfnr-8-4-2
  - Shivappa N, Steck SE, Hurley TG, et al. Designing and developing a literature-derived, population-based dietary inflammatory index. *Public Health Nutr*. 2014;17(8):1689-96. doi: 10.1017/S1368980013002115
  - Kwan A, Marzouk S, Ghanean H, et al. Assessment of the psychometric properties of patient-reported outcomes of depression and anxiety in systemic lupus erythematosus. *Semin Arthritis Rheum*. 2019;49(2) 260-6. doi: 10.1016/j.semarthrit.2019.03.004.
  - de Almeida Macêdo E, Appenzeller S, Lavras Costallat LT. Assessment of the Hospital Anxiety and Depression Scale (HADS) performance for the diagnosis of anxiety in patients with systemic lupus erythematosus. *Rheumatol Int*. 2017;37(12):1999-2004. doi: 10.1007/s00296-017-3819-x.
  - Moustafa AT, Moazzami M, Engel L, et al. Prevalence and metric of depression and anxiety in systemic lupus erythematosus: a systematic review and meta-analysis. *Semin Arthritis Rheum*. 2020;50(1):84-94. doi: 10.1016/j.semarthrit.2019.06.017. Epub 2019 Jun 28.
  - Bai R, Liu S, Zhao Y, et al. Depressive and anxiety disorders in systemic lupus erythematosus patients without major neuropsychiatric manifestations. *J Immunol Res*. 2016;2016:2829018. doi: 10.1155/2016/2829018.
  - Shatri H, Purnamandala, Hidayat R, et al. The correlation of anxiety and depression with C3 and C4 Levels and systemic lupus erythematosus activity. *BMC Psychiatry*. 2023;23(1):785. doi: 10.1186/s12888-023-05285-8.
  - Liu R, Xu H, Guo X, et al. Genomic characterization of two *Escherichia fergusonii* isolates harboring the *mcr-1* gene from the farm environment. *Front Cell Infect Microbiol*. 2022;12:774494. doi: 10.3389/fcimb.2022.774494.
  - Chen Z, Xie Y, Zhou F, et al. Featured gut microbiomes associated with the progression of chronic hepatitis B disease. *Front Microbiol*. 2020;11:383. doi: 10.3389/fmicb.2020.00383.
  - Marciano F, Vajro P. Oxidative stress and gut microbiota. In: Gracia-Sancho J, Salvadó J, editors. *Gastrointestinal Tissue*. Academic Press; 2017. p.113-23. <https://doi.org/10.1016/B978-0-12-805377-5.00008-4>.
  - Zhao Y, Lukiw WJ. Bacteroidetes neurotoxins and inflammatory neurodegeneration. *Mol Neurobiol*. 2018;55(12):9100-7. doi: 10.1007/s12035-018-1015-y.
  - Gibiino G, Lopetuso LR, Scaldaferrri F, et al. Exploring Bacteroidetes: metabolic key points and immunological tricks of our gut commensals. *Dig Liver Dis*. 2018;50(7):635-9. doi: 10.1016/j.dld.2018.03.016.
  - Toumi E, Mezouar S, Plauzolles A, et al. Gut microbiota in SLE: from animal models to clinical evidence and pharmacological perspectives.

- Lupus Sci Med. 2023;10(1):e000776. doi: 10.1136/lupus-2022-000776.
27. Zhang L, Qing P, Yang H, et al. Gut microbiome and metabolites in systemic lupus erythematosus: link, mechanisms, and intervention. *Front Immunol.* 2021;12:686501. doi: 10.3389/fimmu.2021.686501.
  28. Krupa-Kotara K, Gwiozdzik W, Nandzik S, et al. The role of microbiota pattern in anxiety and stress disorders—a review of the state of knowledge. *Psych.* 2023;5:602–18. doi: 10.3390/psych5030038
  29. Zhang Y, Gan M, He Y, et al. Anxiety disorders and gut dysbiosis in primary Sjogren's syndrome-mediated dry eye patients. *Int J Gen Med.* 2023;16:1735–46. doi: 10.2147/IJGM.S405225.
  30. Jacobs JP, Lagishetty V, Hauer MC, et al. Multi-omics profiles of the intestinal microbiome in irritable bowel syndrome and its bowel habit subtypes. *Microbiome.* 2023;11(1):5. doi: 10.1186/s40168-022-01450-5.
  31. Ritz NL, Brocka M, Butler MI, et al. Social anxiety disorder-associated gut microbiota increases social fear. *Proc Natl Acad Sci USA.* 2024;121(1):e2308706120. doi: 10.1073/pnas.2308706120.
  32. Sudo N. Microbiome, HPA axis and production of endocrine hormones in the gut. *Adv Exp Med Biol.* 2014;817:177–94. doi: 10.1007/978-1-4939-0897-4\_8.
  33. Jiang H, Ling Z, Zhang Y, et al. Altered fecal microbiota composition in patients with major depressive disorder. *Brain Behav Immun.* 2015;48:186–94. doi: 10.1016/j.bbi.2015.03.016.
  34. Nguyen TT, Kosciolk T, Maldonado Y, et al. Differences in gut microbiome composition between persons with chronic schizophrenia and healthy comparison subjects. *Schizophr Res.* 2019;204:23–9. doi: 10.1016/j.schres.2018.09.014.
  35. Qing Y, Xu L, Cui G, et al. Salivary microbiome profiling reveals a dysbiotic schizophrenia-associated microbiota. *NPJ Schizophr.* 2021;7(1):51. doi: 10.1038/s41537-021-00180-1.
  36. Mosca A, Leclerc M, Hugot JP. Gut microbiota diversity and human diseases: Should we reintroduce key predators in our ecosystem? *Front Microbiol.* 2016;7:1–12. doi: 10.3389/fmicb.2016.00455.
  37. Chen J, Siliceo SL, Ni Y, et al. Identification of robust and generalizable biomarkers for microbiome-based stratification in lifestyle interventions. *Microbiome.* 2023;11(1):178. doi: 10.1186/s40168-023-01604-z.
  38. Hiippala K, Kainulainen V, Kalliomäki M, et al. Mucosal prevalence and interactions with the epithelium indicate commensalism of *Sutterella* spp. *Front Microbiol.* 2016;7:1706. doi: 10.3389/fmicb.2016.01706.
  39. Van den Abbeele P, Belzer C, Goossens M, et al. Butyrate-producing *Clostridium* cluster XIVa species specifically colonize mucins in an in vitro gut model. *ISME J.* 2013;7(5):949–61. doi: 10.1038/ismej.2012.158.
  40. Zhang P. Influence of foods and nutrition on the gut microbiome and implications for intestinal health. *Int J Mol Sci.* 2022;23(17):9588. doi: 10.3390/ijms23179588.
  41. Kumar A, Pramanik J, Goyal N, et al. Gut microbiota in anxiety and depression: Unveiling the relationships and management options. *Pharmaceuticals (Basel).* 2023;16(4):565. doi: 10.3390/ph16040565.