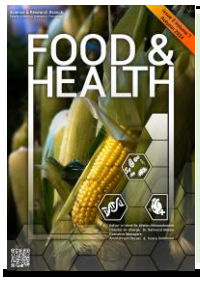

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The Effect of Probiotic Yogurt on Immune System in Systemic Lupus Erythematosus- A Triple-blind, Randomized and Controlled trial

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ABSTRACT

Objective:
Systemic lupus erythematosus (SLE) is an autoimmune multisystem disease. The pathogenesis of SLE is multifactorial, and gut microbiota is one of the important factors in SLE pathogenesis. This research aims to evaluate the impact of probiotic yogurt on autoantibodies, inflammatory markers, and cytokines in SLE patients.

Methods:
In this three-blind, randomized controlled trial, thirty-three lupus patients were divided into two groups. The case group received 200 g of probiotic yogurt, and the control group was given 200 g of simple yogurt for 13 weeks. The patients had SLEDAI ≤6 and were on a stable dose of immunosuppressant in the last 3 months, Demography measurements, Blood sampling, and biochemical measurements were analyzed before and after the intervention.

Results:
The level of autoantibodies such as anti-dsDNA did not change significantly by probiotic yogurt (P=0.733), while anti-SSA increased significantly in the control group (P=0.046). The level of ESR decreased in the cases group (P=0.067), while it increased in the control group (P=0.833), but the difference was not significant. About the effect of probiotic yogurt on IFNα as the main cytokine in SLE, it decreased in the cases group (P=0.809) while it increased in the control group (P=0.067), but the difference was not significant. The probiotic and placebo groups at the baseline demographic and clinical features did not differ significantly.

Conclusions: Probiotic yogurt consumption in SLE patients led to a decrease in IFNα and ESR non-significantly.

This study was registered in the Iranian Registry of Clinical Trials (ID: IRCT20240821062833N1).

1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disorder identified by various autoantibodies, immune complex (IC) formation, and organ inflammation. In addition to the production of autoantibodies, most individuals with SLE show an increased expression of type I interferon (IFN), also recognized as an IFN signature (1). IFNα is a cytokine that can influence various cell types involved in the development of lupus. Various genes within the interferon pathway are linked to the risk of SLE, suggesting that this pathway plays a significant role in the progression of the disease. Lupus patients often exhibit elevated levels of IFNα and increased expression of IFN response genes. The clinical manifestations of lupus can potentially be influenced by IFNα (2). There is an obvious connection between high serum IFNα levels and fever as well as skin rashes (3). SLE affects multiple organs (4), including the kidneys, skin, cardiovascular, musculoskeletal, and central nervous system (5). The etiology and pathogenesis of SLE are not known, though it is approved that genetic susceptibility, sex, ethnicity, and environment help disease onset (6). SLE is characterized by an unpredictable and fluctuating course with the occurrence of frequent relapse and remission and the increase of a big pool of autoantibodies anti-dsDNA (7). In patients suffering from lupus nephritis, the levels of anti-double-stranded DNA)anti-dsDNA(antibodies

in the serum are often associated with systemic lupus erythematosus disease activity index (SLEDAI) scores (8). Anti-dsDNA antibodies can bind to mesangial cells, endothelial cells, and proximal renal tubular epithelial cells to induce cell proliferation, apoptosis, and inflammatory and fibrotic processes (9). Anti-Ro antibody is present in about 50% of patients with SLE (10). The existence of anti-Ro (SSA) antibodies in patients with SLE has been linked to various forms of the illness, such as subacute cutaneous lupus erythematosus, antinuclear antibody-negative lupus, and the lupus-like disease of homozygous C2

and C4 deficiency (11).

The activation of B cells produces a lot of autoantibodies and forms IC with several antigens (12). This activation process can lead to local inflammation and tissue damage due to the deposition of IC, which in turn can intensify the autoimmune response, creating a continuous vicious cycle (13).

Gut dysbiosis may help the pathogenesis of SLE (14). Dysbiosis leads to an alteration in gut barrier function and increases the permeability of intestinal mucosa, which is crucial to preventing the access of pathogens (15). The gut microbiota plays a significant role in modulating systemic inflammation through a variety of interconnected mechanisms: when the integrity of the intestinal barrier is compromised, microbial components such as lipopolysaccharides (LPS) from Gram-negative bacteria can translocate into the bloodstream, triggering systemic inflammation (16). The consumption of probiotics for their health benefits has a lengthy history (17). The therapeutic benefits of probiotics have gained increased attention recently. Research has shown that probiotics have several positive impacts on infectious diseases, diabetes, and gastrointestinal disorders (18). Probiotics can reduce the pathological problems caused by intestinal dysbiosis and restore balance to the microbiota of the digestive tract (19). Lactobacillus and Bifidobacterium are the most well-known probiotics, populations that produce short-chain fatty acids (SCFAs) and work to maintain gut mucosal stability (20).

Probiotic intervention in autoimmune diseases has generated lots of attention as a healthcare strategy for chronic autoimmune disorders like SLE (21). Probiotic bacteria have been claimed to modulate the intestinal microbiota, promoting epithelial healing and preventing bacterial translocation across the epithelium (22). The presence of Lactobacillus spp. Causes reduced renal inflammation in a mouse model of SLE. Consumption of a combination of 5 Lactobacillus spp. Decreases anti-dsDNA levels and proteinuria (23). Considering SLE is characterized by the production of autoantibodies, accumulation of autoreactive and inflammatory T cells, and dysregulated generation of inflammatory cells and pro-inflammatory cytokines, consumption of immunoregulatory probiotics can contribute to the prevention or treatment of autoimmune diseases, mainly improving the inflammatory responses and modulating tolerance in the host to pathogens (24). The present study's objective is to investigate whether the consumption of Lactobacillus Rhamnosus and Bifidobacterium might affect IFNα, anti-dsDNA, anti-RO, C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) levels in SLE patients. So far, the effect of probiotics on IFNα and anti-RO in SLE has not been examined.

2. Materials and methods

2.1. Study subject

This research was carried out as a triple-blind, randomized, and controlled trial. Total subjects

were allocated into two groups by utilizing computer-generated random numbers. We have used simple randomization for allocation. This study involved 33 patients with SLE, having received approval from the Ethics Committees of Tehran University of Medical Sciences (TUMS) (IR.TUMS.DDRI.REC.1399.059), and it was registered in the Iranian Registry of Clinical Trials (ID: IRCT20240821062833N1).

For this research, 33 SLE patients were enrolled from the outpatient clinic of the Rheumatology Research Center (RRC), Shariati Hospital. The consent from each patient was obtained in written form before they participated in the study. An investigation assistant did not participate in the outcome measurements and was responsible for creating the allocation sequence before the research. The participants, physicians, and laboratory personnel were unaware of the allocation of participant groups throughout the trial.

All patients (intervention group: n=19 and control group: n=14) fulfilled the American College of

Rheumatology (ACR) 1997 criteria and the inclusion criteria were defined as follows: age 20 – 60 years, SLEDAI ≤ 6, the body mass index (BMI) ranges from 25 to 40 kg/m2, following a stable medication regimen for the last three months. The exclusion criteria of the study included the use of the probiotic product:

- One month before the study initiation
- Digestive tract disorders or lactose intolerance
- Inflammatory disease including (Pancreatitis, Inflammatory bowel disease (IBD) myocarditis, etc)
- Kidney and liver diseases
- Food supplement consumption at least one month before the intervention
- Smoking
- Lactating or pregnancy
- Changes in the dosage of drugs
- Consumption of alcoholic beverages
- Being on a weight-reduction diet.

2.2. How to prepare and consume probiotic yogurt

For the individual in the probiotic group, 200 g of yogurt was prepared, which contained 106 colony-forming units (cfu) of both Lactobacillus Rhamnosus GG and Bifidobacterium Bifidum (Domino Dairy Industries, Iran) to be used daily for 13 weeks, while the control group was given 200 g/d Yogurt without probiotics for 13 weeks. In the factory, both yogurts were made from one batch with the same compositions and formulations. At the end of the production, probiotic bacteria were added to the sample but not the control. To monitor the stability and viability of the probiotics, we used MRS agar medium to evaluate probiotic bacteria in yogurt samples. Yogurts were distributed among patients once a week. Yogurt labeling has been done by a third person who is unaware of the aim of our study. The yogurts were divided into two groups, A and B. The containers for probiotics and conventional yogurt are the same in appearance.

Two hundred grams of probiotic yogurt contained 114 kcal energy, 0.22 g salt, 9.67 g carbohydrates, 1.5% fat, and 0 g trans fatty acids. In our studies, no adverse effects from the consumption of probiotic yogurt were reported.

2.3. Study design and measurements

Recording of food consumption information, anthropometric measurements, and collection of blood samples were conducted at the baseline and end of the study.

2.4. Assessment of food intake and physical activity

To evaluate the nutritional intake of the patients during the intervention, three 24-hour food diaries were taken from the patients. Food intake was assessed using the Nutritionist IV software, in which the original database is based on the United States Department of Agriculture (USDA) food composition table. The physical activity of the subjects was checked using three days of physical activity recording. It was done in a way that took at least one weekend day. Data from physical activity records were processed using MET-h/day values based on published guidelines.

2.5. Assessment of anthropometric measures

Anthropometric measurements, such as weight and height (Seca, Hamburg, Germany), were assessed before and after the study without shoes and wearing light clothing (using a digital scale to the nearest 0.1 kg). Height was measured using a non-stretched tape (measured accurately to the nearest 0.1 cm). All measurements were taken with Seca equipment from Hamburg, Germany. The BMI was determined by dividing the measured body weight (kg) by the square of body height (m).

2.6. Blood sampling and biochemical measurements

In this study, we assessed the following laboratory data: IFNα, anti-dsDNA, anti-RO, ESR, and CRP in each SLE patient.

A blood sample of 10 ml was collected from participants before and after the intervention. IFNα was measured based on a Biotin double antibody sandwich (Co, Zellbio GmbH, Germany). Serum levels of anti-dsDNA and anti-RO were determined by the chemiluminescent immunoassay (CLIA) method using IDS dsDNA IgG kits and IDS SS-A/Ro kits (Co, Immunodiagnostic Systems, United Kingdom). The method can measure the smallest quantity of analyte, which is referred to as the Limit of Detection (LOD). The value of CRP was determined by the CRP US Kit (Co, Aptec, Belgium), and the ESR level was assessed by an ESR analyzer by the Westergren method (Co, Convergys, Germany).

Sample size calculation

The standard deviations (SDs) for hs-CRP in the control and intervention groups were 5.93. Based on the recommended formula for parallel clinical trials, we calculated a sample size of 17 patients per group (25).

By applying this formula and factoring in a 20% drop-out rate for each group, the required sample size was 20 participants per group.

Statistical analysis

The mean ± standard deviation (SD) or number (%) was used to describe quantitative and categorical variables. After assessing the normality distribution of quantitative variables by the Kolmogorov–Smirnov test, the Independent-t test, or Mann Whitney u test and chi-square or Fisher exact tests were used to compare quantitative and categorical variables between probiotic and placebo groups, respectively. Also, to compare variables before and after intervention, the paired t-test, Wilcoxon tests, ANCOVA, and Quade's ANCOVA were used. The generalized estimating equations (GEE) method was used to adjust the effect of potential confounders. Considering the per-protocol (PP) approach, all analyses were performed using R software, version 4.2.3. P-values less than 0.05 were considered statistically significant.

3. Results

3.1. Demographic and clinical characteristics

Sixty patients were identified in the initial screening. Of thirty-three participants in the present clinical trial, thirty-two were women and one was a man who completed the study (13-week intervention). The mean ages of the probiotic and placebo groups were 43.26 ± 12.5 and 40.36 ± 7.8, respectively. The sex frequency (female) in the probiotic and

the placebo groups was 94.7% and 100%, respectively. The mean duration of disease in the probiotic and the placebo groups was 15.94 ± 8.96 and 11.00 ± 7.66, respectively.

After starting the intervention, a total of 20 participants dropped out, including 8 patients (1 in the probiotic group and 7 in the placebo group) who withdrew from the research due to various reasons like starting antibiotic therapy, pregnancy, and health problems, and 12 patients (7 in the intervention group and 5 in the placebo group) who were excluded from analyses because they had not followed the research protocol, which included had changed their medications, had changed their physical activity level, had surgery, and did not consume yogurt regularly). Thirty- three individuals were randomly assigned to each group. There were no significant differences in the demographic and clinical characteristics at baseline between the probiotic and placebo groups. A comparison of demographic and clinical characteristics of study participants in the 2 experimental groups at baseline and post-intervention was displayed in Table 1.

3.2. Dietary intake and physical activity

The examination of dietary questionnaires and physical activity, which is demonstrated in Table 2, showed that the intervention and control groups did not show significant differences in energy, macronutrient, and micronutrient intakes either at the beginning or at the end of the study ($P > 0.05$). Protein, cholesterol, magnesium, zinc, selenium, and iron intake increased significantly in the intervention group at the end of the study after consumption of probiotic yogurt ($P = 0.007, 0.041, 0.001, < 0.001, 0.026$, and 0.020, respectively). There was no statistical significance for physical activity between the two groups either before or after the study course.

Table 1: Comparison of demographic and clinical characteristics of study participants in the two experimental groups at baseline

Variable	Total n = 33	Probiotic group n = 19 (58%)	Placebo group n = 14 (42%)	P-value
Demographic characteristics				
Age at study onset (Year)	42.03±10.7	43.26±12.5	40.36±7.8	0.412+
Duration of disease (Year)	13.87±8.67	15.94±8.96	11.00±7.66	0.119+
Sex (Female)	32(97.0%)	18(94.7%)	14(100%)	0.999*
BMI (Kg/m^2)	26.23±4.64	26.38±4.3	26.02±5.19	0.830+
Current medication				
Prednisolone	27(81.8%)	16(84.2%)	11(78.6%)	0.999*
Hydroxychloroquine	28(84.8%)	16(84.2%)	12(85.7%)	0.999*
Mycophenolate500	8(24.2%)	6(31.6%)	2(14.3%)	0.416*
Alendronate	8(24.2%)	5(26.3%)	3(21.4%)	0.999*
Pantoprazole	5(15.2%)	4(21.1%)	1(7.1%)	0.366*
Azathioprine	6(18.2%)	4(21.1%)	2(14.3%)	0.490*

+Based on independent-t or Mann-Whitney Tests; *Based on Chi-square or Fisher exact tests.
BMI: Body Mass Index

Table 2: Comparison of nutrition intakes and physical activity in the two experimental groups at baseline and throughout the study

Variable	Probiotic group n = 19 (58%)	Placebo group n = 14 (42%)	P-value	Variable	Probiotic group n = 19 (58%)	Placebo group n = 14 (42%)	P-value
Energy (kcal)				**Cholesterol (mg/day)**			
Baseline	1838.31±149.9	1913.43±188.3	0.211+	Baseline	138.62±61.0	136.01±71.4	0.911+
End of study	1848.21±102.7	1876.43±145.6	0.890‡	End of study	188.55±85.1	158.96±74.5	0.317‡
Change*	9.89±27.54	-37.00±41.01	0.332+	Change*	49.93±22.63	22.95±28.03	0.455+
P-value	0.724†	0.551†	-	P-value	0.041†	0.428†	-

Continues of table 2

Variable	Probiotic group n = 19 (58%)	Placebo group n = 14 (42%)	P-value	Variable	Probiotic group n = 19 (58%)	Placebo group n = 14 (42%)	P-value
Protein (g/day)				**DHA-Omega 3 (g/ day)**			
Baseline	50.83±8.3	51.50±9.4	0.832	Baseline	0.03±0.1	0.02±0.1	0.603
End of study	58.04±8.2	55.33±9.9	0.362	End of study	0.01±0.03	0.01±0.02	0.714
Change*	7.21±2.46	3.83±3.01	0.855	Change*	-0.02±0.02	-0.01±0.02	0.858
P-value	0.007	0.225	-	P-value	0.260	0.168	-
Fat (g/day)				**EPA-Omega 3 (g/day)**			
Baseline	78.15±18.2	86.82±13.5	0.144	Baseline	0.01±0.03	0.006±0.02	0.504
End of study	82.26±10.3	88.81±15.4	0.145	End of study	0.009±0.03	0.003±0.008	0.413
Change*	4.11±5.47	1.99±4.67	0.942	Change*	-0.002±0.01	-0.003±0.01	0.864
P-value	0.463	0.678	-	P-value	0.857	0.887	-
Carbohydrate (g/day)				**Vitamin A (RE/day)**			
Baseline	230.32±30.8	236.66±41.0	0.615	Baseline	525.02±537.4	424.69±235.6	0.743
End of study	224.76±27.0	220.47±36.7	0.655	End of study	544.53±499.5	474.21±361.6	0.895
Change*	-5.56±7.98	-16.19±14.54	0.499	Change*	19.50±172.34	49.52±111.96	0.894
P-value	0.495	0.286	-	P-value	0.999	0.826	-
Diet fiber (g/day)				**Vitamin D (µg/day)**			
Baseline	12.97±2.8	12.73±3.7	0.837	Baseline	0.29±0.2	1.17±3.1	0.477
End of study	13.84±2.5	14.63±2.5	0.359	End of study	0.54±0.57	1.14±2.9	0.560
Change*	0.87±0.86	1.90±0.99	0.423	Change*	0.25±0.15	-0.03±1.20	0.548
P-value	0.327	0.096	-	P-value	0.124	0.778	-
PUFA (g/day)				**Calcium (mg/day)**			
Baseline	26.18±5.7	25.79±4.1	0.855	Baseline	413.32±99.9	429.99±123.4	0.671
End of study	25.83±5.0	25.87±4.2	0.783	End of study	433.29±112.4	475.32±87.5	0.425
Change*	-0.35±1.55	0.08±1.27	0.913	Change*	19.97±39.26	45.33±35.42	0.648
P-value	0.778†	0.950†	-	P-value	0.617	0.198	-
SFAs (g/day)				**Sodium (mg/day)**			
Baseline	15.77±3.5	18.91±6.9	0.122	Baseline	718.16±267.1	757.91±338.4	0.708
End of study	16.22±2.8	18.89±5.2	0.071	End of study	653.51±256.3	832.14±275.1	0.047
Change*	0.45±1.19	-0.35±1.55	0.743	Change*	-64.65±88.15	74.23±124.29	0.355
P-value	0.305	0.822	-	P-value	0.473	0.561	-
Potassium (mg/day)				**Physical activity: MET (h/week)**			
Baseline	1652.79±278.9	1654.00±338.7	0.991	Baseline	38.74±2.9	38.63±3.8	0.923
End of study	1735.63±175.9	1789.64±270.9	0.406	End of study	38.44±2.6	38.39±2.3	0.840
Change*	82.84±65.16	135.64±100.70	0.649	Change*	-0.30±0.60	-0.24±0.91	0.953
P-value	0.220	0.201	-	P-value	0.601	0.797	-

*Change (Post-Pre): Mean ± Standard Error of Mean; † Based on Paired-t or Wilcoxon Tests; +Based on independent-t or Mann-Whitney Tests; ‡ Based on ANCOVA or Quade's ANCOVA (Baseline value was included as a covariate);

; † Based on repeated measure analysis GEE adjusted by iron and sodium change.

Abbreviation: DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; Re: retinol; PUFA: polyunsaturated fatty acid; SFA: saturated fatty acid; MET: metabolic equivalent

3.3. Biochemical measurement

3.3.1. The Influence of Lactobacillus Rhamnosus and Bifidobacterium Bifidum on IFNα serum level

In patients with SLE, serum IFNα2 is a useful indicator for monitoring treatment responses and disease activity (26). This study investigated the effect of Rhamnosus and Bifidobacterium Bifidum on proinflammatory cytokine at the beginning and end of the trial. The outcomes indicated that consumption of probiotic yogurt decreased IFNα levels in the intervention group and increased in the placebo group (P-value = 0.809, P-value = 0.067, respectively), but this difference was not significant.

3.3.2. The Influence of Lactobacillus Rhamnosus and Bifidobacterium Bifidum on anti-dsDNA and anti-RO level

Anti-dsDNA is considered a particular marker for SLE. Due to the high frequency, sensitivity, and specificity, the presence of this autoantibody could be nearly diagnostic for SLE (27). The coexistence of anti-dsDNA and anti-Ro antibodies can provide a more comprehensive understanding of disease activity and potential complications in SLE patients.

To evaluate the effect of the two bacteria on autoantibodies, we measured anti-dsDNA and anti-RO levels in the serum. The results showed that anti-dsDNA levels increased in the probiotic group (69.15 ± 138.88 to 90.21 ± 175.74, respectively), and the placebo group observed a decrease (71.17 ± 164.78 to 68.09 ± 161.03, respectively), but differences were not statistically significant. The level of anti-RO decreased in the probiotic group, but its level increased the statistically significant placebo group (P-value = 0.575, P-value = 0.046, respectively).

3.3.3 .The Influence of Lactobacillus rhamnosus and Bifidobacterium bifidum on ESR and CRP level

CRP and ESR are both non-specific markers of systemic inflammation; they can be increased in SLE flares and infections (28).

The ESR and CRP levels were evaluated before and after intervention. Although there was a difference in the ESR level in the probiotic and placebo groups (P-value = 0.067, P-value = 0.833, respectively), these differences were not significant. Our results showed that CRP level increased in both groups (P-value = 0.545, P-value = 0.157, respectively), but there was no observed significant difference between them (Table 3).

The consumption of probiotics can affect immune responses and have an important role in the improvement of inflammatory responses and the production of autoantibodies.

Table :3 Effect of 13 weeks of probiotic supplementation use on inflammatory biomarkers in patients with systemic lupus erythematosus

biochemical measurements	Groups	Follow up Times		Change	P-value†
		Baseline	End of study		
INF-α (pg/ml)	Probiotic	214.69±75.20	209.40±60.57	-5.29±8.23	0.809
	Placebo	193.14±74.75	214.79±71.97	21.65±10.84	0.067
	P-value	0.382	0.100	0.122†*	-
Anti-dsDNA/titre (Iu/ml)	Probiotic	69.15±138.88	90.21±175.74	21.05±42.48	0.733
	Placebo	71.17±164.78	68.09±161.03	-3.09±4.41	0.541
	P-value	0.594+	0.547‡	0.742	
Anti-Ro (Au/ ml)	Probiotic	137.42±240.61	126.27±234.85	-11.15±10.07	0.575
	Placebo	119.71±239.83	131.49±256.85	11.78±8.03	**0.046**
	P-value	0.450	0.905	0.128	
ESR (mm/1st hr)	Probiotic	26.89±19.16	20.95±18.30	-5.95±4.43	0.067
	Placebo	21.29±19.51	22.29±19.12	1.00±2.63	0.833
	P-value	0.315	0.203	0.265	-
CRP (mg/l)	Probiotic	2.98±2.92	3.24±2.94	0.26±0.33	0.545
	Placebo	1.47±1.12	2.14±2.09	0.67±0.33	0.157
	P-value	0.290	0.747	0.333	-

†* Based on repeated measure analysis GEE adjusted by Iron and Sodium change.

4. Discussion

There is a relationship between the composition of the gut microbiota and the pathogenesis of SLE (29-31). Dysbiosis in the gut microbiota makes autoimmunity triggered via the potential mechanisms of translocation and molecular mimicry. These mechanisms can cause dysregulation in immune cells, specifically an imbalance between T-helper cells /Regulatory T cell (Th17/Treg) cells, as well as dysregulation in cytokines, including an increased expression of type I interferon resulting in the improvement and progression of SLE (32). Lipopolysaccharide (LPS) could induce one possible mechanism to modulate the immune system. It increases in the intestinal microbiome of mice with SLE, and therefore, via the toll-like receptor 4 (TLR4) activation, such as the production of proinflammatory cytokines like tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6), and type I interferons (IFNα, IFNγ), which are elevated in SLE patients and animal models of SLE as well (33). Gut dysbiosis was found in the toll-like receptor 7 TLR7-dependent murine models of SLE, autoimmunity can be driven by the translocation of Lactobacillus reuteri into the liver and secondary lymph organs (34). The TLR7/IFN I pathway plays a considerable role in human SLE (35).

Consumption of probiotics has improved clinical manifestations, decreased pro-inflammatory cytokines, and enhanced regulatory cytokines (36). In SLE patients, normal gut microbiota could be restored and the inflammatory responses could be reduced by consumption of probiotic bacteria like Bifidobacteria and Lactobacillus (21).

In our study, the effects of these two bacteria, Bifidobacterium bifidum and L. rhamnosus, on the levels of IFNα, anti-dsDNA, anti-RO, ESR, and CRP have been investigated, which has not been done in other studies.

In SLE, IFNα is normally overexpressed and related to disease activity and can induce the

hyperactivation of myeloid dendritic cells that subsequently stimulate autoreactive T lymphocytes that participate in SLE pathogenesis (37). The bacterial microbiota can be important in controlling the expression of homeostatic type I IFNs by plasmacytoid dendritic cells (pDCs), which is needed for transcriptional, epigenetic, and metabolic programming of conventional dendritic cells (cDCs) (38).

Vieira et al,. showed that in mice (NZW × BXSB) F1, the translocation of the gut commensal

Enterococcus gallinarum to the liver stimulated type I interferon expression and anti-dsDNA antibody production through the activation of the aryl hydrocarbon receptor (AhR) system (39). According to a report by Zegarra-Ruiz et al., the TLR7.1 transgenic mice, a resistant starch (RS) -rich diet produces SCFA, suppressing the abundance of L. reuteri and reducing lupus symptoms by decreasing pDCs and interferon pathways (34).

In the current study, we investigated whether probiotics could decrease the level of IFN I. Our findings showed a decrease in interferon, but it was not statistically significant because it is probably because of the small sample size or period duration. This research is similar to Zegarra-Ruiz et al study.

In patients with SLE, intestinal dysbiosis increases the production of autoantibodies by B and T cells via the overexpression of TLR9 on peripheral blood mononuclear cells (PBMCs) (40). The modulation of the effector/regulatory T cell equilibrium in non-autoimmune mice was demonstrated through the engagement of TLR9 by commensal DNA. These findings increase the probability that commensals and their nucleic acids might affect immunoregulatory pathways,

which could potentially contribute to the emergence of systemic autoimmunity (41, 42).

In lpr mice, a leaky gut (23) is characterized by decreased expression of tight junction proteins, increased permeability, and enhanced plasma LPS levels. Lupus progression is accelerated by LPS in several lupus-prone mouse models, including NZBWF1 mice, enhancing polyclonal B-cell activation, plasma anti-dsDNA antibodies, and renal failure (43, 44). When probiotics are consumed for a long time, they can neutralize gut microbiota dysbiosis, causing a decrease in antibody production and the suppression of inflammatory responses, ultimately resulting in less severity and reduced symptoms of SLE (45). One research has shown a negative connection between the levels of Synergistetes in the gut and plasma anti-dsDNA antibody, as well as IL-6 levels (14). Another study indicated that the consumption of Lactobacillus fermentum CECT5716 (LC40) reduced the increased plasma anti-dsDNA, endotoxemia, and hypertension in NZB/WF1 mice (46).

Our results opposed other studies, indicating increased anti-dsDNA and decreased anti-Ro in the probiotic group. An investigation by Dhawan et al. revealed that an investigation by Dhawan et al., revealed that circulating ds-DNA levels have increased in humans with hypercholesterolemia as well as hypercholesterolemic mice (47). In our study, the probiotic group was divided into two groups based on the level of anti-dsDNA: increased anti-dsDNA and decreased anti-dsDNA and then those groups were statistically analyzed. Patients who had consumed more cholesterol showed an increase in the level of anti-dsDNA. It seems this increase is not related to probiotic intervention and probiotic consumption does not lead to an increase in anti-dsDNA. Our result confirms Dhawan et al.'s research.

Our study demonstrated anti-RO levels increased significantly in the placebo group. Considering that ESR levels increased in hypercholesterolemic subjects (48), we divided the placebo group into two groups: increased cholesterol and decreased cholesterol and then based on statistical analysis that has been done, in the group with increased cholesterol, ESR levels increased significantly. In addition, one study has indicated mucocutaneous manifestations are associated with

elevation of ESR (49). Furthermore, according to a study by Logar et al., subacute cutaneous lupus erythematosus has been linked to anti-Ro (SSA) antibodies in patients with SLE (50). Our study has shown an increase in consumption of cholesterol leads to an increas level of ESR that may result in increas anti-Ro. Regarding the follow-up period and sample size of our study, we found that anti-Ro did not significantly increase based on the cholesterol level, but it did significantly in the total number. Finally, Anti-dsDNA levels and anti-RO may decrease significantly if the number of colonies and bacterial species diversity increases.

It seems no study has been done on the relationship between anti-Ro and probiotics. The local gut micro-environment is influenced by inflammatory processes, which can alter the microbial composition on the mucosal surface (51). Therefore, the progression of the disease may be regulated by constant interaction between local and systemic autoimmunity, the gut mucosa, and the microbiota (52).

SCFA, which is a secondary metabolite of probiotics, affects several cellular processes like chemotaxis, differentiation, proliferation, and apoptosis, playing crucial roles in inflammation (53, 54). The activation of the G-protein-coupled receptor 43 (GPR43) by SCFAs is a crucial step in the suppression of inflammation, as demonstrated in various mouse illness models for colitis, arthritis, and asthma (55). Hence, the connection between the metabolic function of the gut microbiota and the inflammatory response is provided through the GPR43 binding of short-chain fatty acids, though the exact mechanism is unknown (56). SCFA might suppress inflammation and oxidative stress by blocking the enzymatic synthesis of hepatic CRP (57).

The impact of probiotics on inflammatory parameters like CRP and ESR has been evaluated in other chronic inflammatory diseases like rheumatoid arthritis (RA). The advantages of probiotic supplementation in reducing CRP levels in RA patients have been reported (58), while another study has shown that the inflammatory parameters (CRP and ESR) did not display considerable change in RA patients after probiotic consumption (59).A previous study in SLE patients showed consumption of symbiotic supplementation , high-sensitive c-reactive-protein (hs-CRP) did not significantly increase, while in the placebo group, hs-CRP increased significantly (60), which is consistent with our results. According to our analysis, the level of CRP increased in the probiotic and placebo groups. Our research suggests ESR and CRP levels may decrease significantly if probiotics in SCFAs or supplements are taken. The impact of the intervention of SCFA in humans resulted in the improvement of inflammation through the Treg/Th ratio balance (61).

Strengths and limitations

This is the first clinical trial examining the effects of probiotic yogurt administration on anti-RO and IFNα on SLE patients. The small sample size was due to the limitation. Our research suggests that the sample size of other studies should be increased.

5. Conclusion

Gut microbiota might motivate signs and progress of some autoimmune illnesses. Probiotics have demonstrated useful effects in the control of autoimmune diseases like SLE.

Our research demonstrated that intake of probiotic yogurt for 13 weeks among patients with SLE decreased ESR, IFNα, and anti-RO antibodies, but it was not significant. Some factors like Follow-up periods, increasing the sample size, using a broader range of probiotic strains and doses, and symbiotic supplementation should be considered in future research.

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7. Author contribution

RB: Writing original draft preparation. TF: Design of the study. EH and MM: reviewed, edited, and contributed scientific prospects to the original. AE, TF, EH, MM, and, MA: revising the article critically, and final approval of the article.All authors read and approved the final manuscript.

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8. Conflict of interest

The authors declare that there is no conflict of interest.

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