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ORIGINAL ARTICLE

Comparative analysis of gut microbiota composition in the fecal samples from type 2 diabetes mellitus patients and healthy individuals: a case control study

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ABSTRACT

Background and Objectives: Insulin resistance and elevated blood glucose levels are the hallmarks of Type 2 Diabetes Mellitus (T2DM), a chronic metabolic condition. Emerging research suggests that gut microbiota may play a causal role in T2DM. This study compares T2DM patients' gut microbiota to healthy controls, focusing on *Lactobacillus*, *Bifidobacterium*, *Akkermansia muciniphila*, *Prevotella*, Bacteroidetes, and Firmicutes.

Materials and Methods: This case-control research involved 50 T2DM patients and 50 healthy controls, aged 39-75. Quantitative real-time PCR (qPCR) employing 16S rRNA gene primers was used to detect and quantify bacterial diversity in fecal samples. Statistical analyses were performed to compare the microbiota composition between groups.

Results: The gut microbiome of patients with Type 2 Diabetes Mellitus differed significantly from that of healthy controls. In T2DM patients, *Lactobacillus* spp. and the Firmicutes phylum had higher relative fold differences, while *A. muciniphila* had lower abundance. No substantial alterations were seen in *Bifidobacterium* spp., *Prevotella*, or Bacteroidetes. T2DM patients had more *Lactobacillus* spp. and Firmicutes and less *A. muciniphila* in their gut microbiome.

Conclusion: While gut microbiota is linked to T2DM, this study analyzes the bacterial composition to identify taxa that change significantly. Further research is essential to unravel the complex relationships between gut microbiota and T2DM pathogenesis, particularly through species-level analysis and genomic studies to identify the primary associated clades.

Keywords: Diabetes mellitus; Gut microbiota; Real-time polymerase chain reaction; Case-control

INTRODUCTION

Diabetes is a chronic medical disorder characterized by elevated blood glucose levels and irregular protein and lipid metabolism (1). Blood glucose levels increase due to insufficient secretion by the pancreas or inefficient cellular use of insulin (2). The prevalence of Type 2 Diabetes Mellitus (T2DM) is a serious global health concern linked to the obesity pandemic (3). It continues to rise, alongside the elevated risk of microvascular consequences (retinopathy, nephropathy, neuropathy) and macrovascular (cardiovascular) consequences in individuals with T2DM. Hyperglycemia and other factors related to insulin resistance exacerbate these complications (3). According to data from the International Diabetes Federation, 536.6 million individuals in the 20-79 age range were diagnosed with diabetes in 2021;

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by 2045, this figure is anticipated to rise to 783.2 million (4). Recent research emphasizes the essential role of gut microbiota in the pathophysiology of chronic disease. Dysbiosis, an alteration in gut microbial composition, has been linked to various chronic conditions, including inflammatory bowel disease, diabetes, obesity, and autoimmune disorders (5, 6). The results reveal that some bacterial species exhibit a positive link with fasting glucose and glycosylated hemoglobin (HbA1c), whilst other strains show a negative correlation with HbA1c, fasting glucose, and plasma triglycerides. This suggests that certain bacterial species may affect the association with T2DM (7). The gut microbiome contributes to maintaining intestinal barrier integrity, modulating the immune system, and controlling metabolic functions (8). Microbial products, such as the metabolite trimethylamine N-oxide and the endotoxin lipopolysaccharide, are associated with subclinical inflammatory processes in chronic diseases (5). Dysbiosis affects the interaction between gut microbes and host cells, leading to dysregulation of inflammation that contributes to chronic inflammatory disease pathogenesis (9). Understanding these interactions may give rise to novel disease prevention and treatment strategies. Specifically, dysbiosis of the gut microbiota influences the synthesis of short-chain fatty acids (SCFAs) and alters the bile acid profile. SCFAs have shown several positive impacts on gastrointestinal metabolism in obesity, a condition often associated with diabetes (10).

Recent research reveals the essential role of the gut microbiota in the etiology of T2DM. Studies have identified alterations in the prevalence of particular bacterial genera, including *Bacteroides*, *Proteobacteria*, and Firmicutes, in T2DM patients (11). Studies suggest that gut microbiota regulates metabolism of enteroendocrine cells and the endocannabinoid system, which links low-grade inflammation and metabolic endotoxemia to intestinal barrier integrity (10). Enhanced intestinal permeability is among the characteristics of type 2 diabetes. This condition leads to the transference of microbial products from the intestine to the bloodstream and metabolic endotoxemia (12). Diabetic individuals also exhibit reduced levels of various butyrate-synthesizing microorganisms such as *Akkermansia*, *Clostridiales* spp., *Faecalibacterium prausnitzii*, *Eubacterium rectal*, *Roseburia* intestinalis, and *R. inulinivorans*, and a greater prevalence of *Lactobacillus* spp. (13, 14). Recent findings indicate that extracellular vesicles of *Akkermansia muciniphila* can diminish intestinal permeability by improving tight junction functionality (15). Several studies report a significant reduction of *A. muciniphila* in diabetic patients, indicating its potential as a biomarker for early diabetes diagnosis (10, 12). These microbial changes are linked to insulin resistance and metabolic impairment (16). Metabolites generated by gut microbiota, including bile acids, lipopolysaccharides, and SCFA, have been implicated in T2DM development (17). It has not yet been fully understood whether alterations in microbiota composition actually caused T2DM in individuals or whether microbial dysbiosis merely underpins the development of metabolic disorders (18). Environmental factors, particularly diet, significantly influence gut microbiota composition and, consequently, T2DM risk (19). Emerging therapeutic strategies targeting gut microbiota consist of probiotics, prebiotics, and fecal microbiota transplantation (20). However, challenges remain in fully comprehending the complicated correlation between gut microbiota and type 2 diabetes mellitus pathogenesis. Further research is needed to develop effective microbiota-based interventions for T2DM prophylaxis and therapy.

The purpose of this research is to investigate and compare the arrangement of gut microbiota in fecal samples of patients with T2DM and healthy individuals. By focusing on specific bacterial species, genera, or orders and their relative abundances, this research seeks to elucidate the potential differences in microbial communities associated with T2DM. Understanding these differences may elucidate the involvement of gut microbiota in the pathophysiology of T2DM and highlight potential microbial targets for therapeutic interventions. We utilized quantitative real-time polymerase chain reaction (qPCR) to examine the presence and copy number of *Lactobacillus* spp., *Bifidobacterium* spp., *A. muciniphila*, *Prevotella*, Bacteroidetes, and Firmicutes in fresh fecal samples from T2DM patients and healthy controls.

MATERIALS AND METHODS

Study participants. This case-control research was performed with the endorsement of the Research Ethics Committee of Islamic Azad University, Zanjan Branch. The study involved fifty adult patients with Type 2 Diabetes Mellitus (T2DM) referred to

Dr. Ghobadian's Endocrine Clinic in Zanjan, Iran, and fifty healthy, non-diabetic individuals aged between 39 and 75 years. The study period extended from June to November 2021. An endocrinologist confirmed T2DM diagnosis through an oral glucose tolerance test (OGTT), which measured serum fasting blood sugar at baseline and two hours after the administration of seventy-five grams of glucose diluted in five hundred milliliters of water. All diabetic participants received treatment and monitoring according to a standard medical protocol. Informed written consent was secured from all individuals. Exclusion criteria for this research included: 1) recent antibiotic, prebiotic, or probiotic treatment (in the past month); 2) pregnancy and lactation; 3) a history of acute or chronic diarrhea during the month prior to the trial; 4) acute or chronic inflammatory or infectious disorders; and 5) a history of cardiovascular, renal, hepatic diseases, or other gastrointestinal disorders. Demographic data, including gender, age, familial diabetes history, smoking habits, alcohol intake, height, and weight, were obtained by a standardized survey. The body mass index (BMI) was calculated using the formula: weight (kg) / height (m^2).

Sample collection and laboratory measurements. Fresh fecal samples were obtained from participants in sterile containers and promptly delivered to the Microbiology Lab at 4°C. Samples were then preserved at -80°C until DNA extraction. Venous blood samples were obtained following a 12-hour overnight fast. The serum was extracted from the blood samples and preserved at -80°C until analysis. Serum fasting blood sugar (FBS), oral glucose tolerance test (OGTT) results, total cholesterol (TC), triglycerides (TG), and hemoglobin A1c (HbA1c) were measured. FBS, OGTT, TC, and TG levels were analyzed using an auto-analyzer (Hitachi, Cobas C 311, Roche Diagnostics GmbH). HbA1c levels were assessed via high-performance liquid chromatography (HPLC). The workflow is shown in Fig. 1.

DNA extraction from fecal samples. Total DNA was extracted from 200 mg of stool samples using the GeneAll Exgene™ Stool DNA Kit (GeneAll Biotechnology, Songpa-gu, Seoul, South Korea), following the manufacturer's instructions. The concentration and purity of the extracted DNA were evaluated using a Nanodrop spectrophotometer (ND-1000, Nano-Drop Technologies, Wilmington, DE) at 260 nm and 260/280 nm, respectively. All extracted DNA samples were stored at -20°C until subsequent analysis.

Quantitative real-time PCR (qPCR). Diversity of bacteria in fecal samples from diabetic patients and healthy individuals was analyzed using quantitative real-time PCR (qPCR) with specific primers targeting 16S ribosomal RNA genes. Oligonucleotide primers for the identification of *Lactobacillus* spp., *Bifidobacterium* spp., *A. muciniphila*, *Prevotella*, Bacteroidetes, and Firmicutes were chosen from the literature and confirmed for specificity using nucleotide BLAST at NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Table 1). The primers were synthesized by Metabion (Metabion International AG, Planegg/Steinkirchen, and Germany). A conserved sequence of the 16S rRNA gene, found in all bacteria, functioned as an internal control in qPCR.

Initially, amplification specificity was assessed using conventional PCR, and the amplicons were examined by agarose gel electrophoresis. Real-time PCR was conducted under standardized conditions in a 20 µl reaction mixture comprising 10 µl of RealQ Plus 2x Master Mix Green High ROX™ (Ampliqon, Denmark), 0.3 µM of each primer pair, and 50 ng of extracted DNA in double-distilled water. Assays were performed in duplicate utilizing an Applied Biosystems Step OnePlus™ Real-Time PCR System, with mean results computed. The amplification program consisted of an initial cycle at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 57-60°C (primer dependent, see Table 1) for 30 seconds, and extension at 72°C for 30 seconds. The specificity of amplification in each qPCR run was verified through melting curve analysis. The bacterial concentration in each sample was determined by comparing the threshold cycle (Ct) values with standard curves. These curves were prepared for each qPCR run using 10-fold serial dilutions of *Escherichia coli* ATCC 25922 genomic DNA. The DNA copy number was determined using the formula:

Number of copies= Mass (in grams) × 6.023× 10^{23}/ Average mol. wt. of a base × template length.

Samples exhibiting no fluorescent signal prior to a Ct value of 35 were considered negative. Relative quantification was assessed using the 2^−ΔCt technique and articulated as a relative fold difference in comparison to the reference gene.

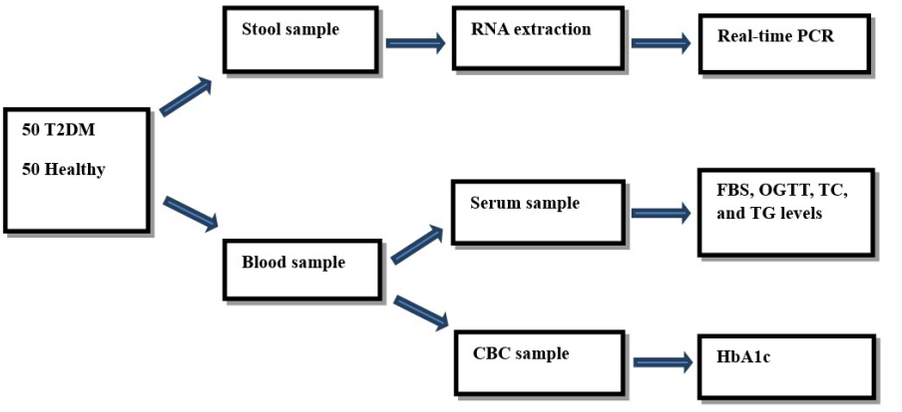

Fig. 1. Sample collection and laboratory measurements flowchart. T2DM: Type 2 diabetes mellitus, FBS: Fasting blood sugar, OGTT: Oral glucose tolerance test, TC: Total cholesterol, TG: Triglycerides, CBC: Complete blood count, HbA1c: Hemoglobin A1c.

Table 1. Sequence of used primers

Target pathogen	Gene	Primer sequence (5-3)	Amplicon size (bp)	Annealing Temperature (°c)
Bifidobacterium spp.	16s rRNA	CGCGTCYGGTGTGAAAG CCCCACATCCAGCATCCA	244	60
Lactobacillus spp.	16s rRNA	GAGGCAGCAGTAGGGAATCTTC GGCCAGTTACTACCTCTATCCTTCTTC	126	60
Prevotella genus	16s rRNA	GGTTCTGAGAGGAAGGTCCCC TCCTGCACGCTACTTGGCTG	121	57
A. muciniphila	16s rRNA	CAGCACGTGAAGGTGGGGAC CCTTGCGGTTGGCTTCAGAT	329	57
Firmicutes phylum	16s rRNA	TGAAACTYAAAGGAATTGACG ACCATGCACCACCTGTC	200	60
Bacteroidetes phylum	16s rRNA	CRAACAGGATTAGATACCCT GGTAAGGTTCCTCGCGTAT	240	60
Universal	16s rRNA	AAACTCAAAKGAATTGACGG CTCACRRCACGAGCTGAC	180	60

Statistical analysis. Statistical analysis was performed utilizing the Statistical Package for Social Sciences (SPSS), version 17.0 (SPSS, Inc., Chicago, IL). Data were expressed as frequencies for qualitative variables and as means ± standard error of the mean (SEM) for quantitative variables. The Mann-Whitney U-test and Fisher's exact test were utilized to evaluate the significance of differences between the two groups. A p-value below 0.05 was considered statistically significant.

RESULTS

Demographic characteristics and biochemical parameters. Table 2 enumerates the demographic and biochemical data of the study participants. The average age of patients with diabetes was 55.8 years, whereas the mean age of the healthy controls was 56.1 years, with no statistically significant difference detected between the two groups ($P > 0.05$). Of the diabetes patients, 30 (60%) were female. No substantial disparity in weight and BMI was noted between diabetic patients and healthy controls ($P > 0.05$). Seventy-four percent of patients with Type 2 Diabetes Mellitus (T2DM) had a familial history of diabetes, compared to just 10% of the control group ($P < 0.05$). Marked disparities in biochemical parameters—including fasting blood sugar (FBS), oral glucose tolerance test (OGTT), and hemoglobin A1c (HbA1c) levels—were observed between diabetic and healthy participants ($P < 0.05$). Nevertheless, no statistically significant differences were detected in total cholesterol (TC).

Table 2. Demographic attributes and biochemical metrics of participants

Variable	Type 2 diabetes (n=50)	Health control (n=50)	P value
Gender n (%)			
Male	20 (40%)	23 (46%)	0.68
Female	30 (60%)	27 (54%)	
Age*(years)	55.8 ± 1.49	56.1 ± 0.6	0.62
BMI*(kg/m^2)	27.2	26	0.10
Family History n (%)	37 (74%)	5 (10%)	0.001
Smoking n (%)	6 (12%)	3 (6%)	0.48
Alcohol consumption n (%)	1 (2%)	0	
FBS*(mg/dL)	147.9	86.8	0.001
OGTT*(mg/dL)	219.2	116.8	0.001
HbA1c*(%)	7.3	4.9	0.001
TC*(mg/dL)	190.4	188.8	0.60
TG*(mg/dL)	191.1	173.5	0.18

Note: *Data are presented as mean ± SD.

P value <0.05 was considered statistically significant.

BMI, body mass index; FBS, fasting blood sugar; OGTT, oral glucose tolerance test; HbA1c, hemoglobin A1c; TC, total cholesterol; TG, triglycerides.

Bacterial presence and quantification. The quantification of various bacterial species revealed notable differences between T2DM patients and healthy controls. *Bifidobacterium* spp. were detected in 98% of participants in both groups, showing no substantial disparity. However, *Lactobacillus* spp. were significantly more prevalent in T2DM patients (92%) compared to healthy controls (66%), with a significant difference in the Ct values (P = 0.04) and higher log copies of DNA per milliliter (8.10 ± 0.73 vs. 7.26 ± 0.80, P = 0.02). Similarly, *A. muciniphila* showed a significant difference in prevalence (90% in T2DM patients vs. 74% in controls, P = 0.04) and lower bacterial load in T2DM patients (6.26 ± 1.19 log copies DNA ml^-1) compared to healthy controls (7.90 ± 1.51, P = 0.02). Firmicutes phylum was also found to have a significantly higher bacterial load in T2DM patients (10.00 ± 0.43 log copies DNA ml^-1) compared to controls (9.28 ± 0.94, P = 0.02), as reflected by significantly lower Ct values (P = 0.002). No significant variations were found in the presence or bacterial load of the *Prevotella* genus and Bacteroidetes phylum between the two groups. The results of this research indicate distinct alterations in the gut microbiota composition of T2DM patients, particularly in the prevalence and quantity of *Lactobacillus* spp., *A. muciniphila*, and Firmicutes phylum (Tables 3 and 4).

Table 3. Presence of bacteria in control and T2DM

	T2DM (n= 50)	Control (n= 50)	P value
Presence of bacteria[a]			
Bifidobacterium spp.			
Yes	49 (98%)	45 (90%)	0.022
No	1 (2%)	5 (10%)	
Lactobacillus spp.			
Yes	49 (98%)	50 (100%)	0.2
No	1 (2%)	0	
Prevotella genus			
Yes	46 (92%)	50 (100%)	0.04
No	4 (8%)	0	
A. muciniphila			
Yes	33 (66%)	37 (74%)	0.38
No	17 (34%)	13 (26%)	
Firmicutes phylum			
Yes	50 (100%)	50 (100%)	>0.05
No	0	0	
Bacteroidetes phylum			
Yes	50 (100%)	50 (100%)	>0.05
No	0	0	

Note: [a]Values recorded as number (percentage), Fisher's exact test.

Table 4. Copy number and Ct values of bacteria in control and T2DM

	T2DM (n= 50)	Control (n= 50)	P value
Range and mean values of Ct			
Bifidobacterium spp.	16.4-38 (24.8)	13.8- 38 (25.7)	0.39
Lactobacillus spp.	19.1-36.4 (26.5)	16.5- 34.1 (28.1)	0.04
Prevotella genus	12.3-38 (18.6)	13.2- 34.9 (18.1)	0.61
A. muciniphila	18.4- 39.6 (31)	13.6-37.8 (27.8)	0.02
Firmicutes phylum	12.9-22.3 (17.2)	12.6-33.7 (19.5)	0.002
Bacteroidetes phylum	13.5-23.7 (18.1)	11.8-30.6 (19.6)	0.06
Log copies DNA ml^{-1} [b]			
Bifidobacterium spp.	8.47 ± 0.88	8.32 ± 1.13	0.45
Lactobacillus spp.	8.10 ± 0.73	7.26 ± 0.80	0.02
Prevotella genus	9.78 ± 1.22	9.87 ± 0.93	0.78
A. muciniphila	6.26 ± 1.19	7.90 ± 1.51	0.02
Firmicutes phylum	10.00 ± 0.43	9.28 ± 0.94	0.02
Bacteroidetes phylum	9.07 ± 1.05	9.23 ± 0.92	0.56

Note: [b]Values recorded as log copies DNA ml^{-1}, Mann–Whitney test.

Relative quantification of bacteria. The quantitation of intestinal bacteria using the 2-ΔCt method revealed significant differences between T2DM patients and healthy controls. *Lactobacillus* spp. exhibited a markedly elevated relative fold difference in T2DM patients (0.0115 ± 0.040) compared to controls (0.0013 ± 0.035, P = 0.04). *A. muciniphila* was significantly lower in T2DM patients (0.0039 ± 0.019) than in controls (0.0512 ± 0.16, P = 0.03). Firmicutes phylum demonstrated a markedly elevated relative fold difference in individuals with T2DM (0.485 ± 0.33) compared to controls (0.252 ± 0.29, P = 0.001). No substantial differences were identified in the relative fold differences of *Bifidobacterium* spp., *Prevotella* genus, and Bacteroidetes phylum between the two groups. These results highlight specific modifications in the composition of gut microbiota linked with T2DM, particularly in the proportional prevalence of *Lactobacillus* spp., *A. muciniphila*, and Firmicutes phylum (Table 5).

DISCUSSION

The host and intestinal bacteria often coexist in a commensal relationship. Nonetheless, gut microorganisms may pose risks when the environment experiences abnormal changes. Dysbiosis of gut bacterial communities in humans or animal models may lead to various chronic disorders, including autism, cancer, diabetes, obesity, and inflammatory bowel disease. Multiple studies have investigated the composition of gut microbiota in individuals with diabetes. However, previous studies have not yielded consistent results, suggesting the necessity of examining the gut microbiota in populations of diverse ethnicities (10, 21, 22).

This research revealed notable disparities in the composition of gut microbiota between T2DM patients and healthy controls. Notably, the presence and abundance of *Lactobacillus* spp., Firmicutes phylum, and *A. muciniphila* varied considerably between the two groups. The mean values of Ct for *Lactobacillus* spp. and Firmicutes phylum were markedly decreased in T2DM patients vs. healthy individuals, indicating higher bacterial load or abundance in the diabetic group (P < 0.05). Specifically, the log10 copies DNA ml-1 for *Lactobacillus* spp. was 8.10 in T2DM patients and 7.26 in healthy controls (P = 0.02), and for Firmicutes phylum, the values were 10.00 in T2DM patients and 9.28 in healthy controls (P = 0.02). Conversely, *A. muciniphila* was significantly less prevalent in T2DM patients, with a mean Ct value indicating a lower bacterial concentration in comparison to the control group (P = 0.02). The log10 copies of DNA ml-1 for *A. muciniphila* were 6.26 in T2DM patients and 7.90 in healthy controls (P = 0.02). These findings indicate that T2DM is associated with distinct alterations in gut microbiota, distinguished by elevated amounts of *Lactobacillus*

Table 5. Quantification of intestinal microbiota in individuals with T2DM and healthy controls. Data are presented as relative fold differences, calculated using the 2-ΔCt method. Values represent the mean ± standard error of the mean (SEM)

	T2DM (n= 50)	Control (n= 50)	P value
Relative difference			
Bifidobacterium spp.	0.0095 ± 0.14	0.0244 ± 0.08	0.23
Lactobacillus spp.	0.0115 ± 0.040	0.0013 ± 0.035	0.04
Prevotella genus	0.371 ± 0.36	0.394 ± 0.33	0.60
A. muciniphila	0.0039 ± 0.019	0.0512 ± 0.16	0.03
Firmicutes phylum	0.485 ± 0.33	0.252 ± 0.29	0.001
Bacteroidetes phylum	0.311 ± 0.27	0.292 ± 0.32	0.32

spp. and Firmicutes phylum, and decreased levels of *A. muciniphila*. The study's findings indicate that T2DM is correlated with distinct alterations in the gut microbiota. The increased prevalence and abundance of *Lactobacillus* spp. and Firmicutes, alongside a reduction in *A. muciniphila*, suggest a dysbiotic state in T2DM patients. This dysbiosis, characterized by shifts in microbial composition, may facilitate the progression of T2DM. The observed variations in gut microbiota between individuals with type 2 diabetes mellitus and healthy controls have considerable implications for clinical practice and public health. The specific microbial patterns observed in T2DM patients could potentially be used as diagnostic biomarkers (23). Monitoring these microbial changes could aid in early detection of T2DM risk or progression. In addition, understanding the specific microbial imbalances associated with T2DM may lead to targeted therapeutics (24). For example, interventions aimed at restoring the gut microbiome to a more balanced state, such as prebiotics, probiotics, or fecal microbiota transplantation, may be investigated (23).

Moreover, tailoring dietary recommendations based on individual gut microbiome profiles could be a promising approach for managing T2DM. Dietary interventions could be designed to enhance the expansion of advantageous bacteria and diminish the prevalence of possibly detrimental bacteria (25).

A study conducted by Ejtahed et al. (2020) found higher levels of *Lactobacillus*, *Prevotella*, and *Escherichia* in Iranian type 1 and type 2 diabetic patients than in healthy controls. However, in their study, no substantial change was identified in *Akkermansia* levels between the diabetic and control groups (10). In contrast to our findings, Remely et al. (2016) and Qin et al. (2012) indicated that the prevalence of *A. muciniphila* increased in the type 2 diabetes group (26, 27).

Research has shown that certain *Lactobacillus* species, including *L. acidophilus* and *L. rhamnosus*, are more prevalent in T2DM patients compared to healthy controls (23, 28). Conversely, some beneficial species like *L. gasseri*, *L. reuteri*, and *L. plantarum* are found in higher numbers in healthy individuals (28). Probiotic interventions using specific *Lactobacillus* strains have demonstrated potential in ameliorating T2DM symptoms. For instance, *L. rhamnosus* LRa05 reduced fasting blood glucose levels, attenuated insulin resistance, and reshaped gut microbiota in diabetic mice (24). Similarly, *L. sakei* Probio65 and *L. plantarum* Probio-093 showed anti-diabetic effects by inhibiting key digestive enzymes, reducing body weight, and adjusting gut microbiota composition in high-fat diet-induced diabetic mice (25). These findings suggest that certain *Lactobacillus* species may have therapeutic potential in managing T2DM.

T2DM patients show reduced proportions of Firmicutes and *Clostridia* in relation to non-diabetic individuals, with the Bacteroidetes to Firmicutes ratio positively correlating with plasma glucose levels (29). Conflicting evidence indicates that a greater abundance of Firmicutes and an elevated Firmicutes to Bacteroidetes ratio correlate with an enhanced lean tissue index in T2DM patients (30). Additionally, T2DM patients with non-alcoholic fatty liver disease (NAFLD) demonstrate reduced levels of Bacteroidetes and Firmicutes in comparison to healthy controls (31). A greater prevalence of Firmicutes correlates with heightened NAFLD severity in patients with T2DM, particularly in males with higher BMI and lower glycated hemoglobin levels (30).

Despite the mentioned relationship with T2DM, we did not observe a substantial variation in the abundance of *Bifidobacterium*, *Prevotella*, and Bacteroidetes in fecal samples between T2DM patients

and the controls. The outcomes of earlier research concerning the bacterial load of *Prevotella* in T2DM patients were controversial. In agreement with our results, Sedighi et al. (2017) demonstrated that the quantity of *Prevotella* in fecal samples of the T2DM patients did not exhibit a statistically substantial difference compared to the control group (21). According to the study by Ejtahed et al. (2020), higher levels of *Prevotella* were noted in Iranian type 1 and type 2 diabetic patients than in healthy controls (10). However, Murri et al. (2013) indicated that the quantity of fecal *Prevotella* was diminished in T2DM patients compared to the control group (32). The outcomes in question may stem from *Prevotella's* opposing influences on glycemic control. It acts as a mucin-degrading bacterium that compromises intestinal integrity, while simultaneously functioning as a succinate producer that inhibits hepatic glucose output by stimulating intestinal gluconeogenesis (10). Also, *Prevotella* levels are linked to a diet rich in carbohydrates and fiber. Therefore, the greater quantity of *Prevotella* in diabetic patients is probably due to long-term high carbohydrate consumption (10).

A. muciniphila, a gut microbe, shows promising potential in managing T2DM and related metabolic disorders. Studies indicate that *A. muciniphila* can improve metabolism, reduce inflammation, enhance intestinal barrier function, and maintain microbiota homeostasis (33). In animal models, *A. muciniphila* administration has been shown to ameliorate liver function, decrease gluco/lipotoxicity and oxidative stress, and normalize gut microbiota in diabetic rats (34). The bacterium's beneficial effects extend to reducing insulin resistance and increasing insulin sensitivity and glucose tolerance (35). Furthermore, *A. muciniphila* has demonstrated the ability to slow down the progression of diabetes, obesity, and inflammatory bowel disease in mice, with preliminary clinical trials in obese and diabetic individuals showing promising results (36). These results indicate that *A. muciniphila* may represent a potential probiotic for managing T2DM and associated metabolic conditions.

This study has limitations that must be acknowledged when evaluating the findings. Firstly, the sample size was limited, comprising just 50 individuals with T2DM patients and 50 healthy controls. This limited sample size may reduce the applicability of the findings to the wider population. Secondly, the cross-sectional study design precludes the determination of causal connections between variations in gut microbiota and T2DM. Longitudinal studies are essential to ascertain if alterations in gut microbiota precede the onset of T2DM or are a consequence of the disease. Additionally, other confounding factors, such as diet, lifestyle, and medication use, were not fully controlled and could have influenced the gut microbiota composition. Future studies should address these limitations by incorporating larger and more diverse populations and utilizing longitudinal designs, and controlling for potential confounders to enhance comprehension of the gut microbiota's role in T2DM.

Future investigation should expand upon the findings of this study and focus on conducting longitudinal studies to confirm the causal link between gut microbiota and T2DM. These studies should aim to track changes in gut microbiota composition over time and assess their impact on the development and progression of T2DM. In addition, it would be valuable to investigate the effects of specific dietary interventions, such as high-fiber or low-carbohydrate diets, on gut microbiota and metabolic outcomes in T2DM patients, as this could provide insights into potential therapeutic strategies. Furthermore, personalized dietary interventions based on individual microbiota profiles could be examined to determine their effectiveness in enhancing glycemic control and reducing insulin resistance. Overall, future research should aim to clarify the processes via which gut microbiota alterations affect metabolic health and identify targeted interventions to modulate the gut microbiota for better management of T2DM.

CONCLUSION

The study observed a clear distinction in the gut microbiota composition between people with Type 2 Diabetes Mellitus and healthy controls. Notably, patients with T2DM exhibited an increased abundance of *Lactobacillus* spp. and the Firmicutes phylum, while *A. muciniphila* was more prevalent in the healthy group. These variations are significant because gut microbiota has been shown to exert influence on host metabolism and immune responses, and the observed differences could thereby facilitate the progression of insulin resistance and systemic inflammation, key features of T2DM.

While these findings are compelling, the study's constraints, encompassing its comparatively limited sample size and cross-sectional methodology, prevent strong conclusions about causality. Therefore, the authors emphasize the need for larger, longitudinal studies to track temporal changes in gut microbiota and to assess the effects of dietary and probiotic interventions on both the microbiome and metabolic outcomes in T2DM patients. These findings highlight the need to explore the complex relationship between gut microbiota and T2DM, which could lead to novel therapeutic approaches.

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