76693_Auto_Edited-check.docx

WORD COUNT	4798	TIME SUBMITTED	01-AUG-2022 09:06PM
		PAPER ID	89061295

Correlation between gut microbiota and glucagon-like peptide-1 in patients with gestational diabetes mellitus

Yun-Yi Liang, Ling-Yu Liu, Yan Jia, Yi Li, Jie-Na Cai, Yi Shu, Jing-Yi Tan, Pei-Yi Chen, Hong-Wei Li, Hui-Hua Cai, Xiang-Sheng Cai

Abstract

BACKGROUND

Gestational diabetes mellitus (GDM) places both the mother and offspring at high risk of complications. Increasing evidence suggests that the gut microbiota plays a role in the pathogenesis of GDM. However, it is still unclear whether the gut microbiota is related to blood biochemical traits, particularly glucagon-like peptide-1 (GLP-1) in GDM patients.

AIM

To explore the correlation between gut microbiota and blood biochemical traits, particularly GLP-1 in GDM patients.

METHODS

The V4 region of the 16S ribosomal ribonucleic acid (rRNA) gene was sequenced from the fecal samples of 35 pregnant women with GDM and was compared to that of 25 pregnant women with normal glucose tolerance (NGT).

RESULTS

The results showed that *Ruminococcaceae_UCG-002*, *Ruminococcaceae_UCG-005*, *Clostridium_sensu_stricto_1*, and *Streptococcus* were more abundant in the NGT group than in the GDM group. *Bacteroides and Lachnoclostridium* were more abundant in the

GDM group than in the NGT group. Spearman's correlation analysis was performed to identify the relationships between genera and blood biochemical traits. *Paraprevotella*, *Roseburia*, *Faecalibacterium*, and *Ruminococcaceae_UCG-002* were significantly negatively correlated with glucose. *Ruminococcaceae_UCG-002* was significantly negatively correlated with hemoglobin A1c. *Bacteroides* was significantly positively correlated with glucose. *Sutterella*, *Oscillibacter*, and *Bifidobacterium* were significantly positively correlated with GLP-1. A random forest model showed that 20 specific genera plus glucose provided the best discriminatory power, as indicated by the area under the receiver operating characteristic curve (0.94).

CONCLUSION

The results of this study reveal novel relationships between the gut microbiome, blood biochemical traits, particularly GLP-1, and GDM status. These findings suggest that some genera are crucial for controlling blood glucose-related indices and may be beneficial for GDM treatment. Alteration in the microbial composition of the gut may potentially serve as a marker for identifying individuals at risk of GDM.

Key Words: Gut microbiome; Glucagon-like peptide-1; Gestational diabetes mellitus; Glucose

Liang YY, Liu LY, Jia Y, Li Y, Cai JN, Shu Y, Tan JY, Chen PY, Li HW, Cai HH, Cai XS. Correlation between gut microbiota and glucagon-like peptide-1 in patients with gestational diabetes mellitus. *World J Diabetes* 2022; In press

Core Tip: Increasing evidence suggests that the gut microbiota plays a role in the pathogenesis of gestational diabetes mellitus (GDM). However, it is still unclear whether the gut microbiota is related to blood biochemical traits, particularly glucagon-like peptide-1 (GLP-1) in GDM patients. To the best of our knowledge, this is the first study to analyze the relationship between GLP-1 and gut microbiota in patients with

GDM, and this is the first report on the relationship between Paraprevotella, Roseburia, Faecalibacterium and glucose in GDM, and the first report on the associations between GLP-1 and genus including Sutterella, Oscillibacter, and Bifidobacterium in GDM.

INTRODUCTION

Gestational diabetes mellitus (GDM) is defined as abnormal glucose tolerance in pregnancy[1]. It is one of the most common complications of pregnancy. The incidence of GDM has increased due to lifestyle changes, increasing maternal age, and changes to the GDM diagnostic criteria. The incidence of GDM is reported to be 13.20%. GDM is closely related to the occurrence of perinatal maternal and neonatal complications, and also significantly increases the risk of long-term metabolic diseases in pregnant women and newborns. The pathogenesis of GDM is complex and has not yet been comprehensively elucidated. Timely diagnosis and intervention are of great significance to the long-term health of pregnant women and their fetuses.

In recent years, with increased research and understanding of gastrointestinal hormones, their roles in the occurrence and development of GDM have attracted growing attention. Research suggests that GDM patients exhibit insufficient glucagon-like peptide-1 (GLP-1) secretion during pregnancy and after delivery relative to their blood glucose level. Bonde *et al*[2] found that the postprandial GLP-1 level of pregnant women was decreased, especially in GDM patients. However, with the recovery of blood glucose homeostasis after delivery, postprandial GLP-1 secretion gradually returned to normal. Kosinski *et al*[3] confirmed that decreased GLP-1 in patients with GDM is reversible. Changes in GLP-1 levels may be related to insulin resistance (IR) as a result of high blood glucose levels. However, it cannot be ruled out that changes in GLP-1 levels may be involved in the occurrence and development of GDM.

Evidence indicates that the gut microbiota is closely related to GDM[4]. The mechanism underlying the effect of probiotics in diabetes has not yet been fully elucidated, but it may be related to reductions in oxidative stress, regulation of the immune response, reductions in inflammation, and regulation of the gut microbiota[5,6].

In addition, probiotics can also reduce postprandial blood lipid levels and improve the absorption of antioxidants, which are related to oxidative stress[7]. Numerous studies have demonstrated that GLP-1 has insulin tropic and antioxidant effects[8-10]. Since GLP-1 and the gut microbiota each play roles in GDM, is there a correlation between the two?

Both clinical and animal studies have reported correlations between changes in the GLP-1 Level and changes in the gut microbiota after gastrointestinal bypass surgery in type 2 diabetes mellitus (T2DM) patients or mice[11]. Therefore, it is speculated that GLP-1 may regulate blood glucose by regulating the number and structure of gut microbiota. Several authors have argued that GLP-1 may play a role in regulating blood glucose by increasing the diversity of the gut microbiota[12] and increasing the proportion of probiotics. Together, the current literature provides a comprehensive explanation of the hypoglycemic mechanism of GLP-1 and a reliable experimental basis for the study of GDM therapeutic targets and therapeutic drugs based on GLP-1. Accordingly, one study found that bifidobacteria improved insulin sensitivity by increasing the production of GLP-1[13].

The rapid increase in the prevalence of GDM in recent years cannot be easily explained by genetic factors; thus, it is hypothesized that environmental factors may play a more important role. The gut microbiota constitutes an important environmental factor. GLP-1, as the most important representative of gastrointestinal hormones, may also be involved in the pathogenesis of GDM. Gastrointestinal microbiota and gastrointestinal hormones share the same root, are inseparable, influence and restrict each other, and jointly participate in the occurrence, development, and prognosis of GDM. Thus, a comprehensive study of the correlations between changes in the gut microbiota and GLP-1 will help to further clarify the pathogenesis of GDM. This is of great significance for the prevention, treatment, and prognosis of GDM, and may provide a novel and sensitive index for the clinical evaluation of GDM.

The rapid increase in the prevalence of GDM in recent years cannot be easily explained by genetic factors; thus, it is hypothesized that environmental factors may

play a more important role. The gut microbiota constitutes an important environmental factor. GLP-1, as the most important representative of gastrointestinal hormones, may also be involved in the pathogenesis of GDM. Gastrointestinal microbiota and gastrointestinal hormones share the same root, are inseparable, influence and restrict each other, and jointly participate in the occurrence, development, and prognosis of GDM. Thus, a comprehensive study of the correlations between changes in the gut microbiota and GLP-1 will help to further clarify the pathogenesis of GDM. This is of great significance for the prevention, treatment, and prognosis of GDM, and may provide a novel and sensitive index for the clinical evaluation of GDM.

MATERIALS AND METHODS

Subjects

Patients were screened for GDM in the obstetric outpatient department according to the GDM diagnostic criteria (2014). Thirty-five patients with GDM were randomly selected from the patients who met the diagnostic criteria for GDM; these patients formed the GDM group. A further twenty-five pregnant women with normal glucose tolerance (NGT) were selected as the NGT group. Each subject provided written informed consent before inclusion in the study. The study was approved and carried out in accordance with the guidelines of the Ethics Committee of Nanhai District People's Hospital of Foshan.

The inclusion criteria were as follows: 18-45 years old, female, any education level. Based on the diagnostic criteria for gestational diabetes (2014), during the 24-28th week of gestation, the 75 g oral glucose tolerance test (OGTT) was used to measure each patient's blood glucose levels before, one hour after, and two hours after consuming sugar. If the patient's blood glucose level reached or exceeded 5.1, 10.0, or 8.5 mmol/L, respectively, they were deemed to have GDM and were eligible for inclusion in the GDM group.

The exclusion criteria were as follows: (1) History of chronic digestive system disorder; (2) history of treatment with GLP-1 analogues or GLP-1 receptor agonists; (3)

history of cardiac, renal, or liver dysfunction; (4) multiple pregnancy; (5) pregnancy-induced hypertension syndrome, placental insufficiency, placenta previa, placental abruption, pelvic or soft birth canal abnormalities, or other pregnancy complications; (6) history of mental disorders; (7) exposure to a large amount of radiation, chemical poisons, or drugs that can affect the fetus during pregnancy; (8) tumor history or history of radiotherapy and chemotherapy within the past six months; (9) participation in other research studies; (10) patients lost to follow-up due to various reasons, including the occurrence of other serious diseases during the study; and (11) consumption of antibiotics or probiotics within one month prior to admission.

Sample collection and testing

Fresh fecal samples were collected from the participants and immediately frozen in a refrigerator at -80 °C. After collection of all samples, they were sent to the Treat Gut company for 16S rDNA sequencing.

Blood samples were collected after fasting and then 1 h and 2 h following consumption of sugar. Plasma glucose (Glu), glycosylated serum protein (GSP), low-density lipoprotein (LDL), uric acid (UA), hemoglobin (HB), total cholesterol (TCH), triglyceride (TG), and high-density lipoprotein (HDL) were determined by a Beckman AU5800 fully automatic biochemical analyzer. Glycosylated hemoglobin A1c (HbA1c) was determined by an ADAMS™ A1c HA-8180 automatic glycosylated hemoglobin analyzer. Insulin (INS), thyroid-stimulating hormone (TSH), and free tetraiodothyronine (FT4) were detected by a Maglumi2000plus automatic chemiluminescence immunoanalyzer.

The active forms of GLP-1 in the plasma samples of patients with and without GDM were measured using a GLP-1 (active) ELISA kit (ELabscience, Wuhan, Hubei Province, China).

Bacterial 16S rRNA gene sequencing

The total genomic DNA of each sample was extracted using a fecal genomic DNA extraction kit (Tiangen company). Sixteen S rDNA sequencing was performed by PCR amplification of V4 variable regions (39 to 297 base pairs), and a purified product library was established. The library construction steps followed the library construction method of the Illumina sequencing platform. The sequencing analysis was as follows. First, the Illumina Miseq 2 × 300bp paired-end sequencing data were analyzed. According to the barcode information, the samples were distinguished. Then, the data were merged, spliced and filtered, and quality control analysis was conducted, including Q20 and Q30 scores. The final clean data were analyzed by operational taxonomic units (OTU) cluster analysis and species taxonomy.

Microbiome data

The data were filtered using Mothur software and clustered into OTUs (species) at a similarity level of 97% using Quantitative Insights into Microbial Ecology (QIIMEv) software verion 1.80 (Houlden *et al*, 2016). Based on the OTU analysis, the Ace, Shannon, observed species, Simpson, Chao1 and J indices were calculated as alpha diversity metrics. To compare the microbial composition between the samples, beta diversity analysis was performed using principal component analysis (PCA) and principal coordinate analysis (PCoA). Analysis of similarities (ANOSIM) was applied to evaluate the statistical significance of differences between the groups. A linear discriminant analysis (LDA) effect size (LEfSe) method was employed to evaluate any differences in the gut microbe between the groups.

Statistical analysis

GraphPad Prism (version 7.0) and R version 3.0.2 (R Foundation for Statistical Computing) were using for statistical analyses. The measurement data are expressed as the mean ± SD. Differences between groups were analyzed using one-way ANOVA. The differences were considered statistically significant at $P < 0.05$. Random-Forest classification was performed for discriminating the samples from different groups using

the R package "random forest". The model was employed for five-fold cross-validation of the relative species abundance profile. Case probabilities were calculated by drawing receiver operating characteristic (ROC) curves.

RESULTS

Characteristics of the study population

GDM was diagnosed in 35 women based on fasting or oral glucose-stimulated hyperglycemia, or a combination of the two. Markers of glucose and insulin homeostasis were higher in the GDM group compared with the NGT group (Table 1). Individuals with GDM also had higher hemoglobin A1C ($P = 0.003$) and fasting blood glucose levels ($P < 0.001$). There were no significant differences in pre-pregnancy body weight, BMI, UA, TCH, TG, HDL, LDL, TSH, and FT4 between the two groups.

OTU distributions

In this study, the OTUs were annotated included 14 phyla, 62 families, and 214 genera of gut microbiota; the similarity among samples was 97% (Figure 1A). The total number of OTUs of the NGT group (at the 97% similarity level) was 652, and for the GDM group, it was 619. Venn diagram showed that 560 OTUs were shared by the NGT and GDM groups (Figure 1B).

Alpha and beta diversities

The observed species index of the GDM group was significantly difference from that of the NGT group (25; $P = 0.044$). The Chao1 richness index of the GDM group was significantly different from that of the NGT group (43; $P = 0.004$). The ACE index of the GDM group differed significantly from that of the NGT group (25; $P = 0.0055$). There were no significant differences in the Shannon, Simpson, and J indices between the GDM group and NGT group (Shannon, $P = 0.65$; Simpson, $P = 0.9$; J, $P = 0.91$; Figure 2A). PCA and PCoA indicated that the gut microbiota in GDM patients differed significantly from that of the NGT subjects. There was no difference in the gut

microbiota structure between the groups (ANOSIM, r = 0.019, P = 0.2232). NMDS cluster analysis indicated marked differences between the GDM patients and NGT subjects (Figure 2B).

Taxonomy

The composition of gut microbiota was difference between the groups at the phylum, family, and genus levels. At the phylum level, *Bacteroidetes*, *Proteobacteria*, *Firmicutes*, *Verrucomicrobia*, *Actinobacteria*, *Fusobacteria*, *Synergistetes*, and *Tenericutes* were common phyla in both the GDM group and NGT group, accounting for 98.81% and 98.58% of the gut bacteria of each group, respectively (Figure 3). The GDM group had a lower abundance of *Firmicutes* (31.4% *vs* 33.2%), *Verrucomicrobia* (0.18% *vs* 1.01%, $P < 0.05$), *Synergistetes* (0.003% *vs* 0.110%, $P < 0.01$), and *Tenericutes* (0.05% *vs* 0.08%), and a higher abundance of *Bacteroidetes* (63.50% *vs* 60.81%), *Proteobacteria* (3.03% *vs* 2.56%), and *Fusobacteria* (0.33% *vs* 0.29%), compared to the NGT group. The ratio of *Firmicutes* to *Bacteroidetes* was decreased in the GDM group compared to the NGT group (0.49 *vs* 0.54).

At the family level, a greater number of different families were identified between groups (Figure 4). Fifty-five and 54 of the dominant families were detected in the GDM group and NGT group, respectively. *Bacteroidaceae* (phylum *Bacteroidetes*), *Prevotellaceae* (phylum *Bacteroidetes*), *Acidaminococcaceae* (phylum *Firmicutes*), *Veillonellaceae* (phylum *Firmicutes*), *Lachnospiraceae* (phylum *Firmicutes*), *Ruminococcaceae* (phylum *Firmicutes*), *Enterobacteriaceae* (phylum *Proteobacteria*), and *Tannerellaceae* (phylum *Bacteroidetes*) had the highest relative abundance in the GDM group. While *Bacteroidaceae*, *Prevotellaceae*, *Acidaminococcaceae*, *Veillonellaceae*, *Lachnospiraceae*, *Enterobacteriaceae*, *Ruminococcaceae*, and *Rikenellaceae* were the eight most abundant families in the NGT group.

The bacterial taxa whose levels differed significantly between the two groups were identified by LEfSE analysis (Figure 4). At the family level, *Atopobiaceae*, *Eggerthellaceae*, *Streptococcaceae*, *Christensenellaceae*, *Clostridiaceae*, *Bifidobacteriaceae*, *Lachnospiraceae*, and

Ruminococcaceae were significantly more abundant in the NGT group than in the GDM group.

At the genus level, bacterial genera exhibited significant differences between the two groups (Figure 5). In the NGT group, *Bacteroides* (phylum *Bacteroidetes*), *Prevotella_9* (phylum *Bacteroidetes*), *Phascolarctobacterium* (phylum *Firmicutes*), *Megasphaera* (phylum *Firmicutes*), *Megamonas* (phylum *Firmicutes*), *Lachnospiraceae* (phylum *Firmicutes*), *Escherichia-Shigella* (phylum *Proteobacteria*), and *Prevotella_2* (phylum *Bacteroidetes*) were the eight most dominant genera. The eight most dominant genera in the GDM group were *Bacteroides* (phylum *Bacteroidetes*), *Prevotella_9* (phylum *Bacteroidetes*), *Megamonas* (phylum *Firmicutes*), *Phascolarctobacterium* (phylum *Firmicutes*), *Lachnospiraceae* (phylum *Firmicutes*), *Megasphaera* (phylum *Firmicutes*), *Prevotella_2* (phylum *Bacteroidetes*) and *Parabacteroides* (phylum *Bacteroidetes*).

Ruminococcaceae_UCG-002, *Ruminococcaceae_UCG-005*, *Clostridium_sensu_stricto_1* and *Streptococcus* were more abundant in the NGT group than in the GDM group ($P < 0.05$). *Bacteroides* and *Lachnoclostridium* were more abundant in the GDM group than in the NGT group ($P < 0.05$). *Prevotella_9*, *Oscillibacter*, *Roseburia*, and *Faecalibacterium* were slightly more abundant in the NGT group than in the GDM group.

Functional profiling of the gut microbiome

The COG categories and KEGG pathways were compared between the GDM and NGT groups. Figure 6A showed that three functional KEGG pathways differed between the GDM group and NGT group, including the glycosphingolipid biosynthesis-globo series, synthesis and degradation of ketone bodies, and renal cell carcinoma pathways. Figure 6B showed that 20 COG categories differed between the GDM group and NGT group, including the phosphotransferase system, galactitol-specific IIC component, metal-dependent proteases with possible chaperone activity, uncharacterized protein, homolog of phage Mu protein gp30, uncharacterized protein conserved in bacteria, Acyl-CoA dehydrogenases, putative virion core protein (lumpy skin disease virus), predicted phosphohydrolase, large-conductance mechanosensitive channel,

uncharacterized conserved protein, uncharacterized protein predicted to be involved in DNA repair, predicted permease, DMT superfamily, nicotinic acid mononucleotide adenylyltransferase, amidases related to nicotinamidase, histone acetyltransferase, plasmid maintenance system antidote protein, uncharacterized conserved protein, DNA polymerase III, alpha subunit, uncharacterized protein conserved in bacteria, antirestriction protein, NA polymerase III, and alpha subunit (gram-positive type).

Correlations between blood biochemical traits and gut composition

Spearman correlation analysis was performed to identify whether the different dominant genera were associated with blood biochemical traits in the second trimester of pregnancy (Figure 7). *Paraprevotella*, *Roseburia*, *Faecalibacterium*, and *Ruminococcaceae_UCG-002* were negatively correlated with Glu ($P < 0.05$). *Ruminococcaceae_UCG-002* was negatively correlated with HbA1c ($P < 0.05$). *Clostridium_sensu_stricto_1*, *Desulfovibrio*, and (*Ruminococcus*)_*torques_group* were negatively correlated with pre-pregnancy body weight ($P < 0.05$). *Phascolarctobacterium* was negatively correlated with HDL ($P < 0.05$). *Ruminococcaceae_UCG-003* and *Faecalibacterium* were negatively correlated with height ($P < 0.05$). *Lachnoclostridium* and *Lachnospiraceae_NK4A136_group* were positively correlated with age ($P < 0.05$). *Bacteroides* was significantly positively correlated with Glu ($P < 0.01$). *Sutterella*, *Oscillibacter*, and *Bifidobacterium* were positively correlated with GLP-1 ($P < 0.05$).

Roseburia was negatively correlated with OGTT (0 h), OGTT (1 h), and OGTT (2 h) ($P < 0.05$). *Faecalibacterium* was negatively correlated with OGTT (0 h) and OGTT (1 h) ($P < 0.05$). *Bacteroides* was positively correlated with OGTT (0 h), OGTT (1 h), OGTT (2 h), and GSP ($P < 0.05$). *Lachnoclostridium* was positively correlated with OGTT (1 h) and OGTT (2 h) ($P < 0.05$). *Sutterella* was positively correlated with GLP-1(0 h), GLP-1(1 h), GLP-1(2 h), and pre-pregnancy BMI ($P < 0.05$). *Oscillibacter* was positively correlated with GLP-1(0 h), GLP-1(1 h), and GLP-1(2 h) ($P < 0.05$). *Bifidobacterium* was positively correlated with GLP-1(0 h), GLP-1(1 h), OGTT (2 h), TG, TCH and ($P < 0.05$).

Gut microbiota–based prediction of GDM

Finally, random forest models were used to assess the ability of the genera abundance profiles to predict GDM status (Figure 8A). Twenty genera plus Glu provided the best discriminatory power, as indicated by the area under the receiver operating characteristic (AUROCC) value of 0.94. The value was higher than that achieved with a model including just 20 genera (the best AUC was 0.828) (Figure 8B). Further, models with 20 genera plus GLP-1, INS, or HbA1c had lower AUROCC values than the model with 20 genera plus Glu. The AUROCC values were 0.81, 0.8288, and 0.8502, respectively.

DISCUSSION

In recent years, the relationships between the gut microbiota and diabetes as well as other endocrine diseases have become research hotspots. Similarly, the characteristics of the gut microbiota among pregnant women with GDM have received widespread research attention. To date, research on GDM has focused on the correlation between the gut microbiota and blood glucose or insulin, but there is still a lack of research on the relationship between the gut microbiota and GLP-1. Many studies have reported that GLP-1 is closely related to the gut microbiota and short chain fatty acids[14-16], and changes in the gut microbiota directly affect the secretion of GLP-1, which, in turn, affects insulin and blood glucose. These are closely related to the occurrence of GDM. Therefore, we focused on the relationship between GLP-1 and the gut microbiota in GDM patients. To the best of our knowledge, it is the first report on the relationship between GLP-1 and the gut microbiota in patients with GDM.

At the phylum level, the abundance of *Firmicutes* in gut microbiota of the GDM group is lower than that in NGT group. *Firmicutes* are known to transform carbohydrates and undigested proteins into short-chain fatty acid (SCFA), producing energy for the host organism. As a crucial SCFA, butyrate participates in the activation of multiple physiological signal pathways, including the proliferation and differentiation of regulatory T cells and anti-inflammatory activities[17,18]. Moreover, the GDM group

exhibited reduced phylum levels of *Verrucomicrobia*, *Synergistetes*, and *Tenericutes* compared to the NGT group. Mucin-degrading bacteria *Verrucomicrobia* contribute to glucose homeostasis and intestinal health, and play a key role in the ineratcion between the host tissues and gut microbiome[19]. The gut microbiota in the GDM patients also exhibited higher abundance of *Bacteroidetes*, *Fusobacteria* and *Proteobacteria* compared to healthy subjects. Proteobacteria is an opportunistic pathogen that creates a major structural imbalance in the gut microbiota of GDM patients. The ratio of *Firmicutes* to *Bacteroidetes* in GDM patients is lower than NGT individuals.

At the family level, *Atopobiaceae*, *Eggerthellaceae*, *Streptococcaceae*, *Christensenellaceae*, *Clostridiaceae*, *Bifidobacteriaceae*, *Lachnospiraceae*, and *Ruminococcaceae* were more abundant in the NGT subjects than in the GDM patients. Zhang *et al*[20] reported that *Ruminococcaceae*, *Bifidobacteriaceae*, *Christensenellaceae*, *Erysipelotrichaceae*, *Peptostreptococcaceae*, and *Eggerthellaceae* were more abundant in the NGT subjects, which is consistent with the current study. In line with the study of Zhang *et al*[21], the current results revealed that *Ruminococcaceae* were more abundant in the NGT group than in the GDM group. However, other studies have observed the opposite result[22,23]. The mechanisms remain unclear.

At the genus level, *Ruminococcaceae_UCG-002*, *Ruminococcaceae_UCG-005*, *Clostridium_sensu_stricto_1*, and *Streptococcus* were more abundant in the NGT group than the GDM group. *Bacteroides* and *Lachnoclostridium* were more abundant in the GDM group than in the NGT group. Kuang *et al*[24] found that the proportion of *Bifidobacterium* in the gut microbiota of GDM pregnant women was significantly reduced, while the proportions of *Bacteroides* and *Klebsiella* were significantly increased. Liu *et al*[25] found that compared with normal pregnant women, the proportion of *Faecalis* in GDM patients was significantly lower, while the proportion of *Prevotella* was significantly higher. In the present study, there were no significant differences in *Bifidobacterium*, *Klebsiella*, and *Prevotella* between the GDM group and NGT group. Shujuan Ma found that *Ruminococcaceae_UCG-002* and *Ruminococcaceae_UCG-005* were reduced in women with GDM[21].

One study found that supplementation with *Lactobacillus rhamnosus* in pregnant women may reduce the prevalence of GDM[21]. Another study showed that additional probiotic supplementation from pregnancy through to 12 mo post-delivery can reduce insulin levels and improve insulin sensitivity[22]. In this study, to identify beneficial bacteria for pregnant women, Spearman's correlation analysis was performed to identify the relationships between genera and blood biochemical traits. *Paraprevotella*, *Roseburia*, *Faecalibacterium* ,and *Ruminococcaceae_UCG-002* were negatively correlated with Glu. *Ruminococcaceae_UCG-002* was negatively correlated with HbA1c. *Clostridium_sensu_stricto_1*, *Desulfovibrio*, and (*Ruminococcus*)*_torques_group* were negatively correlated with pre-pregnancy body weight. *Phascolarctobacterium* was negatively correlated with HDL. *Ruminococcaceae_UCG-003* and *Faecalibacterium* were negatively correlated with height. *Lachnoclostridium* and *Lachnospiraceae_NK4A136_group* were positively correlated with age. Zhang *et al*[21] found that *Ruminococcaceae_UCG-002* was negatively correlated with fasting blood glucose levels. In the study of Crusell, *Clostridium (sensu stricto)* was positively correlated with gestational weight[26]. To the best of our knowledge, no studies have yet reported on the relationships between *Paraprevotella*, *Roseburia*, *Faecalibacterium*, and Glu in GDM. The current findings suggest that these genera are crucial for controlling blood glucose-related indices and may be beneficial for GDM treatment.

GLP-1 and its receptor agonist can promote insulin secretion only when the blood glucose level is elevated[27]. This safety feature makes GLP-1 and its agonist suitable for the treatment of GDM, which requires strict maintenance of blood glucose levels and stable, safe blood glucose regulation. Thus, the current study aimed to examine the correlations between the gut microbiota and GLP-1 Levels, and identify beneficial bacteria that can improve the expression of GLP-1 in patients with GDM, so as to more safely control blood glucose. In the present study, *Sutterella* was significantly positively correlated with GLP-1 (0 h), GLP-1 (1 h), GLP-1 (2 h), and pre-pregnancy BMI. Wang *et al*[28] reported that subjects taking metformin exhibited significantly increased relative abundance of *Sutterella*, whereas liraglutide dosing was associated with a significant

increase in the genus *Akkermansia*. Another study showed that *Sutterella* was associated with C-reactive protein levels[29]. In the current study, *Oscillibacter* was significantly positively correlated with GLP-1 (0 h), GLP-1 (1 h), and GLP-1 (2 h). One study reported that *Cyclocarya paliurus polysaccharides* alleviated type 2 diabetic symptoms by increasing eleven SCFA-producing species, including *Oscillibacter_valericigenes* and *Oscillibacter_ruminantium*[30]. *Oscillibacter* belongs to the Clostridia class of Firmicutes, and in the human gut microbiota, this bacterium grow fermentatively, predominantly producing valerate when grown using glucose as a carbon source[31]. In the current study, *Bifidobacterium* was significantly positively correlated with GLP-1 (0 h), GLP-1 (1 h), TG, TCH, and OGTT (2 h). In the study of Zhao *et al*[32], *Bifidobacterium longum DD98* improved the serum and intestinal cell GLP-1 Levels, which protected pancreatic β-islet cells from damage induced by type 2 diabetes. To the best of our knowledge, this is the first report on the associations between GLP-1 and genera such as *Sutterella*, *Oscillibacter*, and *Bifidobacterium* in GDM.

CONCLUSION

In summary, this study contributes to a better understanding of the relationships between the gut microbiota and blood biochemical traits, particularly GLP-1, in individuals with GDM. The current findings suggest that some genera are crucial for controlling blood glucose-related indices and may be beneficial for GDM treatment. Alteration in the microbial composition of the gut may potentially serve as a marker for identifying individuals at risk of GDM. Future studies combining metagenomics and metabonomics would be of value for improving our understanding of the roles of specific strains and metabolites in patients with GDM and supporting precise prevention and intervention strategies for GDM.

Figure 1 Operational taxonomic units distributions. A: Species tree and distribution of the gut microbial community; B: Venn diagram showing the common or specific operational taxonomic units between the groups. .

Figure 2 Gut microbiota alpha and beta diversity indices of gestational diabetes mellitus. A: Gut microbiota alpha diversity indices of gestational diabetes mellitus. The Observed_species, ACE, Chao1, Simpson, Shannon and J values; B: Principal component analysis score plot based on the relative abundance of operational taxonomic units (97% similarity levels); C: Principal coordinate analysis analysis; D: Similarities analysis; E: Non-metric multidimensional scaling.

Figure 3 Taxonomy. A: The top eight abundant species at the phylum level; B: Different bacteria were compared between each group at the phylum level.

Figure 4 LEfSE analysis was performed to determine which bacterial taxa differed significantly between the groups. ; LDA: Linear discriminant analysis.

Figure 5 Bacterial genera exhibited significant differences between the two groups. A: Heatmap showing the relative total abundance of the first 30 genera; B: Microbial community at the genus level between groups.

Figure 6 KEGG pathways and COG categories were compared between the GDM and NGT groups. A: KEGG pathway; B: COG categories.

Figure 7 Spearman's correlations between different dominant genera and blood biochemical traits. $^{a}P < 0.05$, $^{b}P < 0.01$.

Figure 8 Gut microbiota–based prediction of gestational diabetes mellitus. A: Identification of gestational diabetes mellitus (GDM) markers by random forest models; B: Receiver operating characteristic (ROC) curves of operational taxonomic units-based diagnostic biomarkers for GDM.

Table 1 Clinical variables of gestational diabetes mellitus patients and healthy controls

Characters	Control (25)	GDM (31)	*P* value
Age (yr)	28.42 (3.11)	30.18 (3.26)	0.055
Pre-Body weight (kg)	52.42 (7.68)	55.18 (6.48)	0.165
Height (cm)	160.13 (5.44)	157.88 (4.10)	0.063
Pre-BMI	20.73 (2.85)	22.2 (2.37)	0.054
GLU (mmol/L)	4.49 (0.38)	5.77 (0.95)	3.53×10^{-7}
GLP-1 0 h (ug/L)	67.72 (22.89)	75.45(23.23)	0.223
GLP-1 1 h (ug/L)	75.33 (26.14)	84.34 (19.84)	0.099
GLP-1 2 h (ug/L)	71.75 (23.83)	79.21 (24.20)	0.312
OGTT 0 h (mmol/L)	4.47 (0.39)	5.74 (0.99)	3.53×10^{-7}
OGTT 1 h (mmol/L)	7.77 (1.55)	11.23 (2.95)	3.00×10^{-6}
OGTT 2 h (mmol/L)	6.26 (0.87)	9.64 (3.12)	3.96×10^{-6}
Insulin 0 h (uIU/ML)	9.78 (3.41)	14.03 (15.93)	0.239
Insulin 1 h (uIU/ML)	85.85 (43.99)	64.52 (39.67)	0.061
Insulin 2 h (uIU/ML)	57.51 (39.36)	66.52 (45.21)	0.675
GSP (mmol/L)	1.68 (0.35)	1.97 (0.62)	0.054
HbA1c (%)	5.04 (0.30)	5.79 (1.12)	0.003
UA(umol/L)	279.52(68.47)	263.80(81.27)	0.463
TCH (mmol/L)	5.40 (1.06)	5.35 (1.08)	0.993
TG (mmol/L)	1.82 (0.72)	2.45 (1.54)	0.051
HDL (mmol/L)	2.11 (0.52)	2.08 (0.63)	0.946
LDL (mmol/L)	2.41 (0.89)	2.34 (0.82)	0.739
TSH (uIU/ML)	1.82 (3.43)	1.22 (1.24)	0.337
FT4 (pg/ml)	11.39 (3.27)	11.77 (1.96)	0.598

76693_Auto_Edited-check.docx

ORIGINALITY REPORT

29%
SIMILARITY INDEX

PRIMARY SOURCES

1	www.frontiersin.org Internet	170 words — 3%
2	Ting Chen, Yuqing Zhang, Yiyun Zhang, Chunjian Shan, Yingying Zhang, Kacey Fang, Yankai Xia, Zhonghua Shi. "Relationships between gut microbiota, plasma glucose and gestational diabetes mellitus", Journal of Diabetes Investigation, 2020 Crossref	101 words — 2%
3	academic.oup.com Internet	63 words — 1%
4	"Abstracts", Diabetologia, 2005 Crossref	50 words — 1%
5	Katarzyna Cypryk, Tina Vilsbøll, Iwona Nadel, Joanna Smyczyńska, Jens Juul Holst, Andrzej Lewiński. "Normal secretion of the incretin hormones glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1 during gestational diabetes mellitus", Gynecological Endocrinology, 2009 Crossref	47 words — 1%
6	Www.ijbs.com Internet	47 words — 1%
7	hdl.handle.net Internet	

38 words — 1%

8 Guangyong Ye, Long Zhang, Min Wang, Yunbo Chen, Silan Gu, Keyi Wang, Jianhang Leng, Yujia Gu, Xinyou Xie. "The Gut Microbiota in Women Suffering from Gestational Diabetes Mellitus with the Failure of Glycemic Control by Lifestyle Modification", Journal of Diabetes Research, 2019
Crossref
36 words — 1%

9 link.springer.com
Internet
36 words — 1%

10 "Abstracts of the IDF Congress in Paris 2003", Diabetologia, 2003
Crossref
35 words — 1%

11 www.esp.org
Internet
35 words — 1%

12 Hiroshi Kataoka, Hiroyuki Akagawa, Rie Yoshida, Naomi Iwasa et al. "Impact of Kidney Function and Kidney Volume on Intracranial Aneurysms in Patients with Autosomal Dominant Polycystic Kidney Disease", Research Square Platform LLC, 2022
Crossref Posted Content
33 words — 1%

13 Lingxiong Chai, Qun Luo, Kedan Cai, Kaiyue Wang, Binbin Xu. "Reduced fecal short-chain fatty acids levels and the relationship with gut microbiota in IgA nephropathy", Research Square, 2021
Crossref Posted Content
32 words — 1%

14 microbiomejournal.biomedcentral.com
Internet
28 words — 1%

15 www.wjgnet.com
Internet

26 words — < 1%

16 Xiuying Wang, Kan Xiao, Cheng Yu, Longmei Wang, Tianzeng Liang, Huiling Zhu, Xiao Xu, Yulan Liu. "Xylooligosaccharide attenuates lipopolysaccharide-induced intestinal injury in piglets via suppressing inflammation and modulating cecal microbial communities", Animal Nutrition, 2021
Crossref
25 words — < 1%

17 Cornelia Zetu, Simona Popa, Rubin Munteanu. "Postbariatric Surgery Hypoglycemia and Nutritional Deficiencies: Long-Term Follow-Up", Bariatric Surgical Practice and Patient Care, 2021
Crossref
24 words — < 1%

18 Min Xie, Xue-ling Zhang, Yue Wu, Jia-huan Sun, Wei Yu, Pei-lin Cui. "Study on the Correlation Between Biliary Tract and Intestinal Flora and the Formation of Gallstones", Cold Spring Harbor Laboratory, 2022
Crossref Posted Content
24 words — < 1%

19 Yan Feng, Qi Feng, Xinna Song, Xiaoyan Li, Yinghong Zhang, Jianwei Hu. "Whey protein preloading can alleviate stress adaptation disorder and improve hyperglycemia in women with gestational diabetes mellitus", Research Square Platform LLC, 2020
Crossref Posted Content
24 words — < 1%

20 orbi.uliege.be
Internet
24 words — < 1%

21 Yajie Gao, Haimin Chen, Jialin Li, Shuaijun Ren, Zhenglun Yang, Yuping Zhou, Rongrong Xuan. "Alterations of gut microbiota‐derived metabolites in
23 words — < 1%

gestational diabetes mellitus and clinical significance", Journal of Clinical Laboratory Analysis, 2022
Crossref

22 www.mdpi.com
Internet
23 words — < 1%

23 Hezi Li, Zhilin Luo, Hong Zhang, Nali Huang, Dong Li, Chengwen Luo, Tianhu Wang. "Characteristics of Oral Microbiota in Patients with Esophageal Cancer in China", BioMed Research International
Internet
22 words — < 1%

24 Hongzhi Xu, Qiongyun Chen, Yanyun Fan, Bangzhou Zhang et al. "Fecal microbiota transplantation via capsules relieves ulcerative colitis by improving gut microbiota and serum metabolites", Research Square Platform LLC, 2022
Crossref Posted Content
21 words — < 1%

25 Mirjam Bloemendaal, Joanna Szopinska-Tokov, Clara Belzer, David Boverhoff et al. "Probiotics-induced changes in gut microbial composition and its effects on cognitive performance after stress", Cold Spring Harbor Laboratory, 2020
Crossref Posted Content
21 words — < 1%

26 Lei Xiang, Yanfeng Lou, Lingyu Liu, Yuanling Liu et al. "Gut Microbiotic Features Aiding the Diagnosis of Acute Ischemic Stroke", Frontiers in Cellular and Infection Microbiology, 2020
Crossref
20 words — < 1%

27 Yun Han, Yan-li Zheng, Yu-ping Fan, Man-hua Liu, Xiao-yan Lu, Qian Tao. "Association of adiponectin gene polymorphism 45TG with gestational diabetes mellitus diagnosed on the new IADPSG criteria, plasma
20 words — < 1%

adiponectin levels and adverse pregnancy outcomes", Clinical and Experimental Medicine, 2014
Crossref

28 Na Wu, Jingwei Zhou, Heng Mo, Qing Mu et al. "The gut microbial signature of gestational diabetes mellitus and the association with diet intervention", Cold Spring Harbor Laboratory, 2021 — 19 words — < 1%
Crossref Posted Content

29 Smita Nambiar, Helen Truby, Peter SW Davies, Kimberley Baxter. "Use of the waist-height ratio to predict metabolic syndrome in obese children and adolescents", Journal of Paediatrics and Child Health, 2013 — 19 words — < 1%
Crossref

30 Yao Wang, Zhen Wang, Jingjing Sun, Yeben Qian. "Identification of HCC Subtypes With Different Prognosis and Metabolic Patterns Based on Mitophagy", Frontiers in Cell and Developmental Biology — 18 words — < 1%
Internet

31 daneshyari.com — 18 words — < 1%
Internet

32 Qingyun Li, Xiyu Peng, Eric R. Burrough, Orhan Sahin et al. "Dietary Soluble and Insoluble Fiber With or Without Enzymes Altered the Intestinal Microbiota in Weaned Pigs Challenged With Enterotoxigenic E. coli F18", Frontiers in Microbiology, 2020 — 17 words — < 1%
Crossref

33 rsat.sb-roscoff.fr — 17 words — < 1%
Internet

34 www.researchgate.net — 17 words — < 1%
Internet

35 Mengyang Tang, Mingjuan Luo, Wenqian Lu, Rong Zhang, Wei Liang, Jianfen Gu, Xuemei Yu, Xueli Zhang, Cheng Hu. "Nerve Growth Factor is Closely Related to Glucose Metabolism, Insulin Sensitivity and Insulin Secretion in the Second Trimester: A Case-Control Study in Chinese", Research Square Platform LLC, 2020
Crossref Posted Content
16 words — < 1%

36 Ye Yao, Lijing Yan, Han Chen, Ning Wu, Wenbo Wang, Dongsheng Wang. "Cyclocarya paliurus polysaccharides alleviate type 2 diabetic symptoms by modulating gut microbiota and short-chain fatty acids", Phytomedicine, 2020
Crossref
16 words — < 1%

37 Zhang, Ying, Hao-hang Zhang, Jia-hui Lu, Si-yuan zheng, Tao Long, Ying-tao Li, Wei-zhen Wu, and Fang Wang. "Changes in serum adipocyte fatty acid binding protein in women with gestational diabetes mellitus and normal pregnant women during mid- and late-pregnancy", Journal of Diabetes Investigation, 2016.
Crossref
16 words — < 1%

38 downloads.hindawi.com
Internet
16 words — < 1%

39 Luisa F. Gomez-Arango, Helen L. Barrett, H. David McIntyre, Leonie K. Callaway, Mark Morrison, Marloes Dekker Nitert. "Connections Between the Gut Microbiome and Metabolic Hormones in Early Pregnancy in Overweight and Obese Women", Diabetes, 2016
Crossref
14 words — < 1%

40 ijbnpa.biomedcentral.com
Internet
14 words — < 1%

researchonline.jcu.edu.au

41	Internet	14 words — < 1%
42	www.sciengine.com Internet	14 words — < 1%
43	wco-iof-esceo.org Internet	13 words — < 1%
44	www.pubfacts.com Internet	13 words — < 1%
45	elifesciences.org Internet	11 words — < 1%
46	Aoyun Li, Yan Yang, Songkang Qin, Shenjin Lv, Taihua Jin, Kun Li, Zhaoqing Han, Yongzhu Li. "Microbiome analysis reveals gut microbiota alteration of early-weaned Yimeng black goats with the effect of milk replacer and age", Microbial Cell Factories, 2021 Crossref	10 words — < 1%
47	Peng Ju Liu, Yanping Liu, Liangkun Ma, Ai Min Yao, Xiao Yan Chen, Yi Xuan Hou, Li Ping Wu, Liang Yu Xia. " The Predictive Ability of Two Triglyceride-Associated Indices for Gestational Diabetes Mellitus and Large for Gestational Age Infant Among Chinese Pregnancies: A Preliminary Cohort Study ", Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy, 2020 Crossref	10 words — < 1%
48	rep.bioscientifica.com Internet	10 words — < 1%
49	Honggang Yin, Junhao Huang, Min Hu. "Moderate-Intensity Exercise Improves Endothelial Function	9 words — < 1%

by Altering Gut Microbiome Composition in Rats Fed a High-Fat Diet", Journal of Nippon Medical School, 2022
Crossref

50 Shuang Li, Bin Su, Qiu-Shui He, Hao Wu, Tong Zhang. "Alterations in the oral microbiome in HIV infection: causes, effects and potential interventions", Chinese Medical Journal, 2021 — 9 words — < 1%
Crossref

51 op.niscair.res.in — 9 words — < 1%
Internet

52 www.dovepress.com — 9 words — < 1%
Internet

53 www.spandidos-publications.com — 9 words — < 1%
Internet

54 Bao Jiang, Junfeng Zhang, Xiubin Sun, Chunyan Yang, Guanghui Cheng, Mengru Xu, Siyuan Li, Lina Wang. "Circulating exosomal hsa_circRNA_0039480 is highly expressed in gestational diabetes mellitus and may be served as a biomarker for early diagnosis of GDM", Journal of Translational Medicine, 2022 — 8 words — < 1%
Crossref

55 Cheng LONG, Koen Venema. "Pretreatment of Rapeseed Meal Increases Its Recalcitrant Fibre Fermentation and Alters the Microbial Community in an in Vitro Model of Swine Large Intestine", Research Square, 2020 — 8 words — < 1%
Crossref Posted Content

56 Leyu Wang, Hexin Zhang, Hongjie Lei. "Phenolics Profile, Antioxidant Activity and Flavor Volatiles of Pear Juice: Influence of Lactic Acid Fermentation Using Three Strains in Monoculture and Binary Mixture", Foods — 8 words — < 1%
Internet

57	Louise E Olofsson, Fredrik Bäckhed. "The Metabolic Role and Therapeutic Potential of the Microbiome", Endocrine Reviews, 2022 Crossref	8 words — < 1%
58	Qiong WEI, Zilin SUN, Yue YANG, Hong YU, Hongjuan DING, Shaohua WANG. "Effect of a CGMS and SMBG on Maternal and Neonatal Outcomes in Gestational Diabetes Mellitus: a Randomized Controlled Trial", Scientific Reports, 2016 Crossref	8 words — < 1%
59	Roberts, M.D.. "The genome and proteome of coliphage T1", Virology, 20040105 Crossref	8 words — < 1%
60	Rongmei Tang, Jian Yi, Shuangying Lu, Bowei Chen, Baiyan Liu. "Therapeutic Effect of Buyang Huanwu Decoction on the Gut Microbiota and Hippocampal Metabolism in a Rat Model of Cerebral Ischemia", Frontiers in Cellular and Infection Microbiology, 2022 Crossref	8 words — < 1%
61	X. Ling. "Detecting gene clusters under evolutionary constraint in a large number of genomes", Bioinformatics, 01/19/2009 Crossref	8 words — < 1%
62	assets.researchsquare.com Internet	8 words — < 1%
63	docplayer.net Internet	8 words — < 1%
64	pubmed.ncbi.nlm.nih.gov Internet	8 words — < 1%

65 www.science.gov
Internet
8 words — < 1%

66 Lei Liu, Jiajin Hu, Ningning Wang, Yang Liu, Xiaotong Wei, Ming Gao, Yanan Ma, Deliang Wen. "A novel association of CCDC80 with gestational diabetes mellitus in pregnant women: a propensity score analysis from a case-control study", Research Square, 2019
Crossref
7 words — < 1%

67 Simeng Qin, Weiqiang Bai, Todd J Applegate, Keying Zhang et al. "Dietary resistant starch ameliorating lipopolysaccharide-induced inflammation associated with alteration in gut microbiome and glucagon-like peptide 1 signaling", Research Square Platform LLC, 2022
Crossref Posted Content
7 words — < 1%

68 Ya-Shu Kuang, Jin-Hua Lu, Sheng-Hui Li, Jun-Hua Li et al. "Connections between the human gut microbiome and gestational diabetes mellitus", GigaScience, 2017
Crossref
7 words — < 1%

69 Hao Ren, Xiaodong Su, Hanxun Bai, Yuntian Yang et al. "Specific enrichment of microbes and increased ruminal propionate production: the potential mechanism underlying the high energy efficiency of Holstein heifers fed steam-flaked corn", Research Square Platform LLC, 2019
Crossref Posted Content
6 words — < 1%

70 J. Yi, H. Jin, R. Zhang, S. Zhang, P. Chen, X. Yu, X. Zhang. "Increased serum 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF) levels are associated with glucose metabolism in Chinese pregnant women", Journal of Endocrinological Investigation, 2017
Crossref
6 words — < 1%

EXCLUDE QUOTES	OFF	EXCLUDE SOURCES	OFF
EXCLUDE BIBLIOGRAPHY	OFF	EXCLUDE MATCHES	OFF