RESEARCH

The characteristics of intestinal flora in overweight pregnant women and the correlation with gestational diabetes mellitus

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Abstract

Objective: To investigate the characteristics of intestinal flora in overweight pregnant women and the correlation with gestational diabetes mellitus (GDM).

Methods: A total of 122 women were enrolled and divided into four groups according to their pre-pregnancy BMI and the presence of GDM: group 1 (n = 71) with a BMI <24 kg/m², without GDM; group 2 (n = 27) with a BMI <24 kg/m², with GDM; group 3 (n = 17) with a BMI ≥24 kg/m², without GDM; and group 4 (n = 7) with a BMI ≥24 kg/m² with GDM. Feces were collected on the day that the oral glucose tolerance test was conducted. The V3–V4 variable region of 16S rRNA was sequenced using the Illumina Hiseq 2500 platform, and a bioinformatics analysis was conducted.

Results: There were differences between the four groups in the composition of intestinal flora, and it was significantly different in group 4 than in the other three groups. Firmicutes accounted for 36.4% of the intestinal flora in this group, the lowest among the four groups, while Bacteroidetes accounted for 50.1%, the highest among the four groups, making the ratio of these two bacteria approximately 3:5, while in the other three groups, this ratio was reversed. In women with a BMI <24 kg/m², the insulin resistance index (homeostatic model assessment for insulin resistance (HOMA-IR)) in pregnant women with GDM was higher than in those without (P = 0.026).

Conclusion: The composition of the intestinal flora of pregnant women who were overweight or obese before pregnancy and suffered from GDM was significantly different than women who were not overweight or did not suffer from GDM.

Introduction

Gestational diabetes mellitus (GDM) is one of the most common complications during pregnancy, and the incidence rate is increasing every year and is currently approximately 17.5% in China (1). GDM is harmful to the long- and short-term health of the mother and the child. However, the etiology of GDM remains unclear. It may be caused by insulin resistance and pancreatic β-cell secretion defects caused by genetic and environmental factors, but recent studies have revealed that patients who are obese or have type 2 diabetes also have an imbalance in their intestinal flora (2). In particular, the composition of Firmicutes and Bacteroidetes, the two dominant bacteria in the intestinal flora, change in these patients (3). Therefore, the relationship between intestinal flora and GDM has become a focus of recent research.
This study aims to investigate the characteristics of intestinal flora in pregnant women who were overweight before pregnancy and the correlation between intestinal flora composition and GDM. The aim is to explore the pathogenesis of GDM and provide a basis for the health management of pregnant women who are overweight or obese.

Method

Subjects

A total of 122 pregnant women who received antenatal care in the Obstetrics Clinic of the Shanghai First People’s Hospital between September 2019 and June 2020 were enrolled in the study.

Inclusion criteria: (1) residents of Shanghai who ate a diet common to the Songjiang District of Shanghai; (2) no diagnosis of diabetes before pregnancy; and (3) normal glucose tolerance in the first trimester of pregnancy.

Exclusion criteria: (1) multigravida; (2) diabetes, hypertension, thyroid disease, gastrointestinal or cardiovascular diseases, or other internal surgical diseases present before pregnancy; (3) the use of assisted reproductive technology; or (4) a history of antibiotic use ≤2 months prior to enrollment.

The study was approved by the ethics committee of the Shanghai General Hospital of the Shanghai Jiao Tong University School of Medicine (No: 2020KY098), and all patients provided signed informed consent (clinical registration number: ChiCTR2000036575). The research flowchart is shown in Fig. 1.

Criteria and grouping

According to the recommended standard of the Chinese Guidelines for the Prevention and Control of Overweight and Obesity in Adults, a BMI ≥24.0 kg/m² but <28.0 kg/m² indicates that the patient is overweight, while a BMI ≥28.0 kg/m² indicates that they are obese (4). Pre-pregnancy BMI = weight (kg)/height (m²).

A diagnosis of GDM can be confirmed using a 75 g oral glucose tolerance test (OGTT), with blood glucose levels...
when fasting and 1 and 2 h after ingesting the glucose of 5.1, 10.0, or 8.5 mmol/L, respectively (5).

The subjects were divided into four groups according to their pre-pregnancy BMI and the presence of GDM: group 1 (n = 71) with a BMI < 24 kg/m², without GDM; group 2 (n = 27) with a BMI < 24 kg/m², with GDM; group 3 (n = 17) with a BMI ≥ 24 kg/m², without GDM; and group 4 (n = 7) with a BMI ≥ 24 kg/m², with GDM.

Homeostasis model assessment insulin resistance
HOMA-IR = fasting plasma glucose (mmol/L) × FINS (U/mL) / 22.5.

Collection of fecal samples and the extraction, amplification, database construction, and bioinformatics analysis of fecal DNA
On the same day as the OGTT was conducted, 2–3 g (or four scoops) of fresh feces were collected with a sampler, soaked in a preservation solution, and stirred with a sampling spoon to create a uniform mixture. The mixture was then stored at −80°C by a specially assigned person after admission.

Total bacterial DNA was extracted from the fecal samples using a magnetic fecal DNA extraction kit, and the quality of the extracted DNA was detected using a Qubit dsDNA HS Assay Kit (Carlsbad, CA, USA). After eliminating the samples that did not meet the test standard, the V3–V4 variable region of the 16S rRNA was specifically amplified by PCR. PCR products were detected using 2% agarose gel electrophoresis and sequenced using the Illumina Hiseq2500 system, after which a gene library was established. The Ribosomal Database Project classifier Bayesian algorithm was used in the Trimmomatic software to classify the operational taxonomic unit (OTU) representative sequences with a 97% similarity level. The community composition of the samples was analyzed at all levels (kingdom, phylum, class, order, family, genus, and species) and clustered into OTUs before being compared with data in the SILVA rRNA database. The species classification information was annotated. Using the USEARCH software platform, the samples were subjected to bioinformatics analysis, including relative species abundance, OTUs (species richness), and diversity (Ace, Chao, Shannon Diversity, and Simpson’s Diversity index). Pattern recognition analysis based on a forward feature selection combined with linear discriminant analysis was performed using R version 3.5.1.

Statistical analysis
The data were analyzed using the SPSS 22.0 statistical software. Normally distributed measurement data were expressed as mean ± S.D. (x ± S.D.), and the data of different groups were compared using an independent sample t-test. Non-normally distributed measurement data were expressed as mean and interquartile range and were compared using a Mann–Whitney U or χ² test. P < 0.05 / 4 = 0.0083 was considered statistically significant. Spearman’s rank correlation test was used for correlation analysis between different groups at the phylum or genus levels.

Results
Clinical data
There were no significant differences in age, gestational age, gravidity, birth order, or systolic and diastolic blood pressure between the four groups (see Table 1).

Changes in intestinal flora composition
The comparison of the relative abundance of phylum and genus levels of intestinal flora between the four groups
At the phylum level, 22 phyla were found in the intestinal flora of the 122 subjects. The five most common phyla of average abundance were Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, and Verrucomicrobia. The composition ratio of intestinal flora at the phylum level in the four groups is shown in Fig. 2A. At the genus level, 315 genera were found in the intestinal flora of the subjects. The eight most common genera of average abundance were Bacteroides, Faecalibacterium, Megamonas, Ruminococcaceae_UCG002, Bifidobacterium, Other_A, Lachnolocustidium, and Escherichia-Shigella. The composition ratio of intestinal flora at the genus level in the four groups is shown in Fig. 2B.

As shown in Fig. 3, when compared with groups 1, 2, and 3, in group 4, Firmicutes accounted for 36%, which was significantly lower than in the other groups (54, 57, and 56%, respectively); Bacteroidetes accounted for about 50.1%, which was significantly higher than in the other groups (31, 30, and 31%, respectively); and Bacteroides accounted for 48%, which was also significantly higher than in the other three groups (24, 25, and 25%, respectively). The percentage of Megamonas in the patients in group 3 was higher than in the other three groups.
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The comparison of the composition of intestinal flora between the four groups

1) Groups 1 and 2: There was no significant difference in the composition of intestinal flora between pregnant women with and without GDM.

2) Groups 3 and 4: The relative abundance of Firmicutes was significantly lower in group 4 than group 3 ($P = 0.017$), and the relative abundance of Bacteroidetes and Bacteroides was significantly higher ($P = 0.027$ and 0.02, respectively).

3) Groups 1 and 3: There was no difference in the composition of intestinal flora at the phylum level between group 1 and 3. However, the relative abundance of Megamonas was significantly higher in group 3 than group 1 ($P = 0.025$).

4) Groups 2 and 4: The relative abundance of intestinal Firmicutes and Ruminococceae_Ucg002 was lower, and the relative abundance of Bacteroidetes and Bacteroides was higher in group 4 than in group 2 ($P = 0.015$, 0.039, 0.022, and 0.016, respectively; see Table 2).

Table 1 The characteristics of 122 pregnant women.

<table>
<thead>
<tr>
<th></th>
<th>Pre-BMI &lt;24 kg/m²</th>
<th>Pre-BMI ≥24 kg/m²</th>
<th>H value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (± s.d., years)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Pre-BMI &lt;24 kg/m²</td>
<td>30.6 ± 4.47</td>
<td>30.5 ± 3.96</td>
<td></td>
</tr>
<tr>
<td>Pre-BMI ≥24 kg/m²</td>
<td>31.7 ± 5.13</td>
<td>30.7 ± 4.79</td>
<td></td>
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<tr>
<td>H value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age (M(P25–P75) (weeks)</td>
<td>25.55 (25–26)</td>
<td>25.44 (25–26)</td>
<td></td>
</tr>
<tr>
<td>Gravidity (M(P25–P75))</td>
<td>2.14 (1–3)</td>
<td>2.07 (1–3)</td>
<td></td>
</tr>
<tr>
<td>Parity (M(P25–P75))</td>
<td>0.45 (0–1)</td>
<td>0.44 (0–1)</td>
<td></td>
</tr>
<tr>
<td>Systolic pressure (µs.d., mmhgl)</td>
<td>106.61 ± 9.41</td>
<td>112.67 ± 8.54</td>
<td></td>
</tr>
<tr>
<td>Diastolic pressure (µs.d., mmhgl)</td>
<td>69.20 ± 5.84</td>
<td>70.29 ± 12.79</td>
<td></td>
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</tbody>
</table>

Clinical characteristics, biochemical and hormonal variables of GDM and normoglycemic pregnant women at 24–28 weeks gestation are presented as mean ± s.e.m. when normally distributed or median with 25–75th interquartile range. HOMA-IR, HOMA-IR = FPG (mmol/L) × FINS (µU/mL) / 22.5.

Figure 2
Histogram of the distribution of intestinal flora in 122 pregnant women at the phylum and genus levels.
The comparison of the diversity and OTUs of intestinal flora

It was found that, no matter what a patient’s BMI and blood glucose level were before pregnancy, the abundance and diversity (Ace index, Chao index, Shannon index, and Simpson index) of the intestinal flora in the four groups were similar, and none of the differences between the groups were statistically significant. Similarly, there was no significant difference between the four groups in the total number of OTUs (see Table 3).

Identification of the biomarkers of intestinal flora at the genus level in the four groups

Linear discriminant analysis effect size was used to identify the biomarkers of intestinal flora at the genus level. The results revealed that the four groups had different marker florae, as shown in Fig. 3. The marker florae in group 1 were Clostridiales and Deinococcales, and the marker flora in group 2 was Ruminococcaceae_UCG014 and in group 3 was Rahnella. In group 4, there were nine marker florae, and Deinococcus was the characteristic marker from the phylum level to the genus level, but the markers in group 4 also included Obscuribacterales, Clostridium_sensu_stricto_3, and Terrisporobacter.

The correlation between the relative abundance of flora and the biochemical metabolic indexes of the four groups

The correlation analysis of the dominant intestinal bacteria and the glucose metabolism indexes of the women in all
Table 2  The microbiomes composition in phylum and genus levels of four groups are different at 24–28 week gestation.

<table>
<thead>
<tr>
<th></th>
<th>Pre-BMI &lt; 24 kg/m²</th>
<th>Pre-BMI ≥ 24 kg/m²</th>
<th>T value or Z value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-GDM (n = 71)</td>
<td>GDM (n = 27)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Firmicutes (m ± s.d.)</td>
<td>0.54 ± 0.19</td>
<td>0.57 ± 0.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteroidetes (m ± s.d.)</td>
<td>0.31 ± 0.20</td>
<td>0.30 ± 0.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteobacteria ((M(P25–P75))</td>
<td>0.10 (0.02–0.12)</td>
<td>0.09 (0.01–0.09)</td>
<td>0.08 (0.15–0.99)</td>
<td>0.10 (0.006–0.149)</td>
</tr>
<tr>
<td>Actinobacteria ((M(P25–P75))</td>
<td>0.26 (0.003–0.026)</td>
<td>0.02 (0.003–0.024)</td>
<td>0.32 (0.05–0.35)</td>
<td>0.11 (0–0.029)</td>
</tr>
<tr>
<td>Verrucomicrobia ((M(P25–P75))</td>
<td>0.009 (0–0.002)</td>
<td>0.007 (0–0.006)</td>
<td>0.10 (0–0.007)</td>
<td>0.007 (0–0.003)</td>
</tr>
<tr>
<td>Bacteroides ((M(P25–P75))</td>
<td>0.24 (0.074–0.405)</td>
<td>0.25 (0.081–0.433)</td>
<td>0.25 (0.096–0.378)</td>
<td>0.48 (0.25–0.71)</td>
</tr>
<tr>
<td>Faecalibacterium ((M(P25–P75))</td>
<td>0.16 (0.07–0.23)</td>
<td>0.18 (0.06–0.24)</td>
<td>0.15 ± 0.12</td>
<td>0.10 ± 0.08</td>
</tr>
<tr>
<td>Megamonas ((M(P25–P75))</td>
<td>0.018 (0–0.003)</td>
<td>0.053 (0–0.081)</td>
<td>0.095 (0–0.16)</td>
<td>0.031 (0–0)</td>
</tr>
<tr>
<td>Ruminococcaceae_UCG002 ((M(P25–P75))</td>
<td>0.013 (0–0.013)</td>
<td>0.025 (0–0.022)</td>
<td>0.007 (0–0.011)</td>
<td>0.003 (0–0.005)</td>
</tr>
<tr>
<td>Bifidobacterium ((M(P25–P75))</td>
<td>0.015 (0.001–0.012)</td>
<td>0.012 (0.008–0.017)</td>
<td>0.011 (0.001–0.016)</td>
<td>0.010 (0–0.029)</td>
</tr>
<tr>
<td>Other_A ((M(P25–P75))</td>
<td>0.156 (0.09–0.20)</td>
<td>0.122 (0.08–0.18)</td>
<td>0.115 (0.06–0.15)</td>
<td>0.108 (0.08–0.14)</td>
</tr>
<tr>
<td>Lachnoclostridium ((M(P25–P75))</td>
<td>0.022 (0.004–0.03)</td>
<td>0.022 (0.006–0.022)</td>
<td>0.022 (0.005–0.034)</td>
<td>0.015 (0.003–0.015)</td>
</tr>
<tr>
<td>EscherichiaShigella ((M(P25–P75))</td>
<td>0.025 (0–0.003)</td>
<td>0.009 (0–0.008)</td>
<td>0.014 (0–0.01)</td>
<td>0.041 (0–0.12)</td>
</tr>
</tbody>
</table>

Z₁, T₁, and P₁: group 1 compared with group 2; Z₂, T₂, and P₂: group 3 compared with group 4; Z₃, T₃, and P₃: group 1 compared with group 3; Z₄, T₄, and P₄: group 2 compared with group 4.
<table>
<thead>
<tr>
<th>Group</th>
<th>Pre-BMI ≥ 24 kg/m²</th>
<th>Pre-BMI &lt; 24 kg/m²</th>
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<tbody>
<tr>
<td>Non-GDM (n=71)</td>
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<tr>
<td>GDM (n=7)</td>
<td></td>
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<tr>
<td>T value or Z value</td>
<td>T value or Z value</td>
<td></td>
</tr>
<tr>
<td>Z = -0.20, T = 0.06, p = 0.84, Z = -0.54, T = 0.06, p = 0.96</td>
<td>Z = -0.20, T = 0.06, p = 0.84, Z = -0.54, T = 0.06, p = 0.96</td>
<td></td>
</tr>
<tr>
<td>Z = -0.52, T = 0.01, p = 0.69, Z = -0.52, T = 0.01, p = 0.69</td>
<td>Z = -0.52, T = 0.01, p = 0.69, Z = -0.52, T = 0.01, p = 0.69</td>
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<tr>
<td>Z = -0.37, T = 0.00, p = 0.98, Z = -0.37, T = 0.00, p = 0.98</td>
<td>Z = -0.37, T = 0.00, p = 0.98, Z = -0.37, T = 0.00, p = 0.98</td>
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</tr>
</tbody>
</table>

**Table 3.** The α diversity and OTUs of gut microbiome in four groups.

Four groups is shown in Fig. 3, with the correlation at the phylum level shown in Fig. 4A and the genus level shown in Fig. 4B.

In group 4, the relative abundance of *Firmicutes* was negatively correlated and *Bacteroidetes* was positively correlated with 1hPIN levels, and the relative abundance of *Actinomycetes* was positively correlated with 2hPIN levels. The relative abundance of *Verrucomicrobia* was positively correlated with HDL, and *Tenericutes* was negatively correlated with glycaemic hemoglobin (HbA1C) levels.

The relative abundance of *Bacteroides* and *Faecalibacterium* was positively correlated with FINS and HDL, respectively. The relative abundance of *Ruminococcaceae_UCG014* was negatively correlated with HbA1C, FPG, FINS, 1hPIN, 2hPIN, HOMA-IR, and TG. The relative abundance of *Incertae-Sedis* was negatively correlated with FPGs, and the relative abundance of *Eubacterium_coprostanoligenes_group*, *Ruminococcaceae_UCG002*, and *Christensenellaceae_R7_group* were positively correlated with 2hPG. The relative abundance of *Ruminococcaceae_UCG002* was negatively correlated with FINS and 1hPIN levels, and *Christensenellaceae_R7_group* and *Ruminococcus_1* was positively correlated with HDL. However, the relative abundance of *Hafnia* was negatively correlated with HDL.

**Discussion**

Intestinal flora have rich functions and are symbiotic with the host. They not only participate in the routine digestive process by assisting with the digestion and absorption of nutrients but also maintain the intestinal biological barrier by antagonizing the colonization of and infection by pathogenic microorganisms. They can also stimulate and regulate the cellular and humoral immunity of the human body. There are many species of intestinal flora, but they are mainly composed of *Firmicutes, Bacteroidetes, Proteus, Actinomycetes, Fusobacteria*, and *Verrucomicrobia*. The first four of these account for 98% of the total intestinal flora, and *Firmicutes* and *Bacteroidetes* account for 64 and 23%, respectively (1). They are therefore the main components of intestinal flora.

Obesity, a sedentary lifestyle, eating habits, and the use of antibiotics can all lead to an imbalance in intestinal flora (6). When the balance of *Firmicutes* and *Bacteroidetes* is changed, obesity and diabetes may occur. In the intestinal flora of obese mice, *Firmicutes* has been found to decrease by 50%, while *Bacteroidetes* increases by 50% (3). A study of obese children revealed that this phenomenon also exists in humans, with the percentage of *Firmicutes* in their intestinal flora increasing and *Bacteroidetes* decreasing (7).
The present study found that Firmicutes and Bacteroidetes were the two dominant florae in the intestinal flora of pregnant women. However, in pregnant women with GDM who were overweight or obese, the relative abundance of Bacteroidetes was significantly higher and the relative abundance of Firmicutes was lower than in women who were not overweight and women who were overweight but did not suffer from GDM. Firmicutes accounted for 36.4% of the intestinal flora in group 4, which was the lowest of the four groups, while Bacteroidetes accounted for 50.1%, which was the highest of the four groups. The ratio of the two bacteria in this group was approximately 3:5, but in the other three groups, this ratio was reversed.

A previous study on the structural changes of intestinal flora in people with type 2 diabetes found that the relative abundance of Firmicutes decreased and of Bacteroidetes and Proteus increased significantly (8). In addition, the percentage of Bacteroidetes and Firmicutes has been found to be significantly positively correlated with a decrease in glucose tolerance (9). Bacteroidetes has approximately 20 genera, of which Bacteroides is the most abundant in the human gastrointestinal tract (10). A previous study involving 345 patients with type 2 diabetes found that high levels of Bacteroidetes were positively correlated with the occurrence of diabetes (11).

The possible mechanisms of intestinal flora imbalance leading to obesity and diabetes are: (1) changes to Firmicutes and Bacteroidetes in the intestine can lead to an increase in the total heat absorbed from food, thus decreasing the energy lost in stools (12, 13, 14); (2) the interaction of intestinal flora with environmental and genetic factors in patients with diabetes can lead to an increase in intestinal permeability and a change in mucosal immune response, thereby leading to the progression or deterioration of diabetes (15); and (3) the intestinal microbiota are also responsible for energy production through the anaerobic decomposition of dietary fiber, protein, and peptides to produce short-chain fatty acids (SCFAs). Escherichia coli produces the most acetate, propionate, and butyrate (16). Acetate and propionate are primarily produced by Bacteroidetes, and butyrate by Firmicutes. Cao et al. (17) believed that SCFAs, especially butyrate, can reduce the inflammatory response of adipocytes and improve insulin sensitivity and secretion by stimulating the secretion of peptide 1, such as glucagon-1 (18, 19, 20). When the number of Firmicutes producing butyrate decreases, the beneficial effect is greatly weakened.

Obesity is associated with insulin resistance. The present study found that patients in group 3 and 4 had higher HOMA-IR levels. There was no difference in insulin resistance, but there was a significant difference in the composition of the intestinal flora between these two groups. In groups 1 and 2, the basic levels of HOMA-IR were low, and the level of insulin resistance in group 2 was significantly higher than in group 1. However, there was no significant difference in the composition of intestinal flora between these two groups. Therefore, in pregnant women with a normal pre-pregnancy weight, insulin resistance may be an important cause of the increase in blood glucose, but for pregnant women who are overweight or obese before pregnancy, the imbalance of intestinal flora and metabolic changes may be an important mechanism affecting the occurrence of GDM.

These results indicate that for pregnant women with BMI ≥24 kg/m², the regulation of intestinal flora may effectively improve abnormal glucose metabolism and prevent the occurrence of GDM. One intervention choice for metabolic diseases is probiotics, which can safely and effectively alter the intestinal flora. For example, some species of Bifidobacteria and Lactobacillus have significant benefits in regulating the intestinal microbiota, as they can create a beneficial intestinal environment and help maintain a healthy immune system (21, 22). Previous studies have found that improvement in the symptoms of type 2 diabetes, such as improved intestinal integrity, reduced systemic lipopolysaccharide levels, reduced endoplasmic reticulum stress, and improved peripheral insulin sensitivity, can be observed after intervention with probiotics (23, 24, 25). In addition, changing the dietary pattern can help regulate the intestinal flora, for example, avoiding a high-fat diet can improve the diversity of intestinal microflora (26), while a high-carbohydrate diet can increase the abundance of intestinal flora (13) and the levels of Bifidobacterium (27).
Figure 4
Correlation between the intestinal flora at the phylum and genus levels and the blood glucose metabolism indexes of 122 pregnant women. Correlation analysis of the clinical blood glucose metabolism indexes and the characteristic bacteria. In Spearman correlation analysis, *P < 0.05 and ** indicates P < 0.01.
In addition, fecal microbiota transplantation (FMT), a method of transplanting healthy individual flora into a patient’s colon to reconstitute the intestinal flora, can be used, and 6 weeks of FMT treatment in patients with metabolic syndrome has been found to improve insulin sensitivity and increase the number of butyrate-producing intestinal microorganisms (28).

In summary, this study focuses on the characteristics of the flora composition in pregnant women with different pre-pregnancy BMIs when they are diagnosed with GDM. In women with normal blood glucose levels, irrespective of the BMI, the composition of the intestinal flora is similar. The converse is also true, that is, in women with a normal BMI, regardless of the blood glucose level, the composition of the flora is also similar. However, in pregnant women who are overweight and have GDM, the flora composition is significantly different from those with a normal BMI and GDM. The HOMA-IR of pregnant women in group 2 was significantly higher than in group 1 and 3, and this implies that the HOMA-IR of women in group 4 is similarly higher than in group 1 and 3. In addition to the effect of insulin resistance, an unbalanced intestinal flora composition in pregnant women, especially the ratio of Firmicutes to Bacteroides, influences glucose metabolism, raising blood sugar. GDM in pregnant women with a normal BMI is primarily caused by insulin resistance.

However, there are some deficiencies in this study. First, since our hospital did not perform OGTT tests on all patients before their pregnancy, women with prior impaired glucose tolerance may be included. This is a huge limitation to the results, and the research results need to be verified by more rigorous experiments in the future. Second, the study only covered the period before the second trimester, rather than the whole pregnancy, so it was not possible to know the dynamic composition of the intestinal flora of pregnant women throughout their pregnancy. Follow-up studies should extend this study to include the composition of microflora in the first trimester, the third trimester, and the postpartum period. Third, the patients included in this study were all from Shanghai, and the food culture in Shanghai is unique, which may lead to differences in the composition of intestinal flora when compared with other regions. Further research is needed to verify whether the results are applicable to other regions or countries.

**Conclusion**

The present study found that pregnant women with GDM who were overweight or obese prior to pregnancy had an obvious imbalance in intestinal flora. In particular, the relative abundance of Bacteroidetes was significantly increased, while the relative abundance of Firmicutes was significantly decreased when compared with women with a normal pre-pregnancy BMI. This may be an important mechanism for GDM in pregnant women with high pre-pregnancy BMIs. Therefore, clinical practice should offer supplementary probiotics as an intervention and focus on improving the dietary habits of pregnant women who are overweight or obese. FMT treatment can also be carried out, if necessary, thereby reducing the risk of GDM.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

**Funding**

Three year action plan for promoting clinical skills and clinical innovation ability of municipal hospitals (No. SHDC2020CR2060B). The funding body had no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

**Ethics approval and consent to participate**

This study was conducted with approval from the Ethics Committee of Shanghai General Hospital, Shanghai Jiao Tong University school of Medicine (No. 2020KY096). Written informed consent was obtained from all patients.

**Consent for publication**

All participants signed a document of informed consent.

**Availability of data and materials**

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

**Author contribution statement**

D Y Z, X P G, Y N C, and D C C conceived the idea and conceptualized the study. F F L, X M X, and W Y L collected the data. W Y L, F F L, and X M X analyzed the data. Y S, L C, and H K W drafted the manuscript, then Y S and H K W reviewed the manuscript. All authors read and approved the final draft.

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