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Research Article

CORRELATION BETWEEN FOOD QUALITY AND MICROBIOTA PROFILE OF PREGNANT WOMEN IN THE CITY OF PADANG

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ABSTRACT

Background:
Pregnancy is a complex physiological period that requires systemic adaptation of the body. During pregnancy, a balanced blood microbiota plays an important role in maintaining the mother's immune system and supporting fetal development. This study aims to determine the correlation between food quality and the microbiota profile of pregnant women in Padang City.
Methods: This is an observational study with a cross-sectional design. The study was conducted from January to March 2025 at the Centre for Diagnostic and Research on Infectious Diseases (PDRPI) Laboratory, Andalas University. The sample size was 20 participants, selected using total sampling. Microbiota profiling was performed using 16S rRNA metagenomic sequencing. The results of the microbiota profile analysis were analyzed using Microbiome Analysist software.
Results: Statistical analysis showed a p-value of 0.14702 and a t-statistic of -1.5182, indicating that there was no statistically significant difference between food quality and microbiota profile. The comparison of the Firmicutes/Bacteroidetes ratio (F/B ratio) was 1.06, indicating a balanced microbiota in pregnant women.
Conclusion: Although not statistically significant, these differences in microbiota composition are biologically important and suggest a potential association between dietary patterns and systemic health in pregnant women.

Keywords: **Diet, quality, Microbiota, profile, Pregnant, women**

INTRODUCTION

Pregnancy is a complex physiological period that requires systemic adaptation of the body, including significant changes in the mother's immune and metabolic systems. The increase in hormones such as estrogen, progesterone, hCG, and placental lactogen leads to reduced insulin sensitivity and changes in body fat composition, as well as triggering mild inflammatory phenomena that influence foetal growth(1). These changes not only affect hormonal balance and organ physiology but also impact the composition of the mother's microbiota. For decades, microbiota were thought to be limited to the gastrointestinal tract, oral cavity, and skin. However, recent research shows that microbiota can also be found in human blood, and their presence has important relevance to health conditions, including during pregnancy(2).

Blood microbiota are communities of microorganisms in the bloodstream and can serve as biological markers in various clinical conditions. During pregnancy, a balanced blood microbiota is crucial for maintaining the mother's immune system and supporting foetal development. However, an imbalance in the microbiota, or dysbiosis, is suspected to be associated with conditions such as pregnancy-related anaemia, systemic inflammation, and obstetric complications(3). Unlike the gut microbiota, which functions locally, the blood microbiota is part of the circulatory system and directly interacts with immune components such as leukocytes and cytokines. Blood microbiota is thought to play a role in systemic immunomodulation, influencing inflammatory responses, and may even be involved in regulating important biological processes during pregnancy adaptation. A general overview of blood microbiota profiles will provide a better understanding of this potential role in normal physiological conditions(4).

A study conducted by Castillo et al. (2019) reported that the blood microbiota profile in pregnant women undergoes minimal changes between trimesters but remains stable in healthy pregnancies. This study found that the level of microbiota diversity (α-diversity) remains high in healthy pregnant women, and microorganisms such as Streptococcus, Lactobacillus, and Corynebacterium have a protective role for the mother's immune system(2). A study by Amar et al. (2013) supports the notion that blood microbiota has the potential to serve as a systemic biomarker of mild chronic inflammatory status, including during pregnancy. The study found that in individuals with metabolic disorders, blood microbiota tends to be dominated by Proteobacteria and Enterobacteriaceae, which are indicators of abnormal immune responses. If this occurs in pregnant women, it is likely to affect foetal development(5).

Several studies have shown that blood microbiota is not merely a contaminant, but rather an active biological system that can reflect systemic health status, including during pregnancy. However, research on blood microbiota in pregnant women remains very limited, especially in developing countries such as Indonesia. Most studies on microbiota in pregnant women have focused on gut, vaginal, or oral microbiota, while blood microbiota remains a very new and under-explored field.

However, blood, as a systemic medium, plays a crucial role in reflecting the body's overall immunological and inflammatory conditions. Through the bloodstream, various immune mediators such as cytokines, chemokines, and immune cells are circulated throughout the body, including

to the maternal-placental environment. Therefore, analysing the microbiota in the blood of pregnant women could be a potential approach to comprehensively understanding immune and inflammatory status during pregnancy. Recent studies have even shown that microorganisms or their genetic fragments (such as extracellular vesicles or cell-free bacterial DNA) can be detected in blood and are associated with subclinical inflammatory conditions or pregnancy complications like preeclampsia and preterm labour (6). This indicates that although the quantity and diversity of microbiota in blood are relatively low compared to other tissues, their presence still holds significant clinical and pathophysiological relevance. This study aims to investigate the correlation between dietary quality and the microbiota profile of pregnant women in the city of Padang.

MATERIAL AND METHODS

This study has been approved by the Ethics Committee of the Faculty of Public Health, University of Andalas, with research ethics number B/94/UN16.12.D/PT.01.00/2024. The type of study is observational with a cross-sectional design. The study was conducted from January to March 2025 at the Central Diagnostic and Research Laboratory for Infectious Diseases (PDRPI) of the University of Andalas. The sample used in this study was the same as in the previous study, consisting of 20 samples using total sampling technique. A study by Kembel et al (2012) showed that 20 samples are sufficient to characterise >75% of the variation in the dominant microbiome community, particularly in beta-diversity and environmental diversity analyses (7).

Data Collection Process

In this stage, the researchers followed the manual provided with the kit used (Qiagen Extraction Kit):

***1. Sample* Collection**

Sample collection in this study was conducted using blood samples from a previous study. The blood samples from the previous study were stored at the Biomedical Laboratory of Andalas University in 2022. The blood samples were stored in 3 ml EDTA tubes with purple caps. These blood samples were stored at -80°C to ensure their quality was maintained. Subsequently, 20 blood samples were taken, labelled, and stored at -80°C until DNA isolation was performed.

2. DNA Isolation of Samples

DNA isolation was performed manually by the researcher with the assistance of a laboratory assistant. The samples were removed from the -80°C freezer and isolated using the QIAamp DNA Fast Stool Mini Kit (QIAGEN, Germany). The isolation process included lysis, binding, washing, and elution.

3. DNA concentration testing

The isolation results are immediately tested using the Qubit 4 Fluorometer (Thermo Fisher Scientific, USA), following the Qubit dsDNA assay protocol. The success of the isolation process is determined by measuring the DNA concentration using the Qubit dsDNA assay. The minimum concentration taken is 5 ng/µl.

4. Library preparation

Library preparation is constructed according to the Illumina kit protocol. PCR amplification products are processed for fragmentation and adapter ligation using

Nextera XT. The steps PCR 1, PCR clean-up 1, PCR 2, and PCR clean-up 2 are performed according to the 16S Library Preparation Protocol. All samples that have been cleaned up are pooled into one tube. Pooled samples were quantified using the high-sensitivity Qubit dsDNA assay kit on a Qubit fluorometer (Invitrogen, USA), and fragment sizes were analysed in the Biorad CFX96 Touch Real-Time PCR Detection System. Pooled libraries were prepared for sequencing.

5. qPCR

Samples meeting the Qubit 4 fluorometer concentration criteria will proceed to qPCR analysis. qPCR procedures follow the kit protocol. Instrument settings at each stage—Predenaturation, Denaturation, Annealing, and Extension—and temperatures at each stage are adjusted according to the 16S Library Preparation Protocol for the Illumina MiSeq system (Illumina, USA).

6. Sequencing

Pooled samples are normalised to a concentration of 4 nM and denatured with 5 L of 0.2 N NaOH. A 1.2 pM library was added with 1% PhiX control (PhiX Control v3, Illumina, USA) and sequenced on the Illumina MiSeq platform (Illumina) using the Mid-Output MiSeq System Kit (600 cycles). The sequencing process followed the MiSeq system guide and was guided by trained Illumina technicians.

Microbiome profiling analysis was performed using Microbiome Analysis software. If the alpha diversity index (p-value <0.05) was significant, it indicated a meaningful relationship between food quality and microbiome profile.

RESULTS

The Overview of Microbiota Profile

Based on the research conducted, a comparison of food quality with the microbiota profile at the phylum and genus levels is shown in the figure below

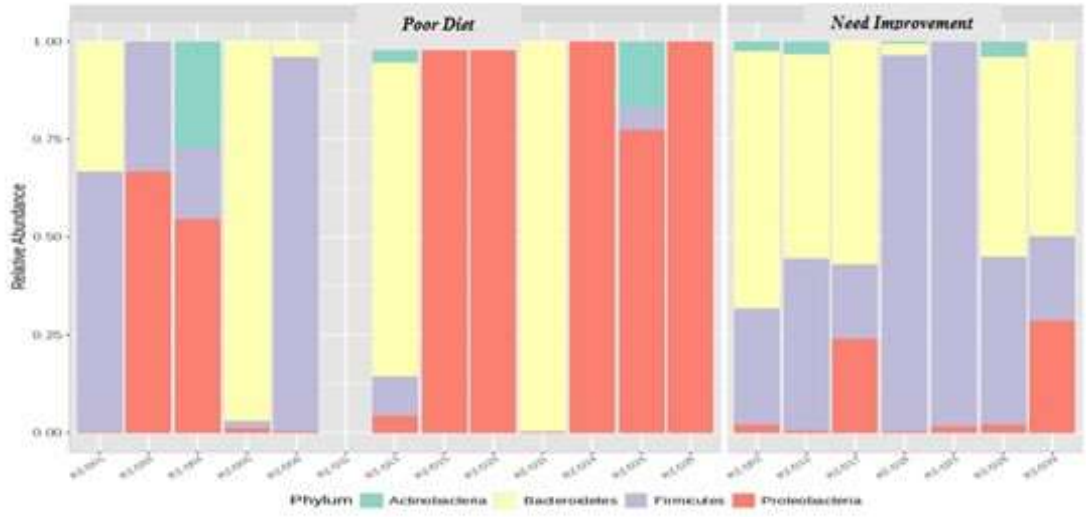

Figure 1. Comparison of Microbiota Profiles of Pregnant Women's Diet Quality at the Phylum Level

As shown in Figure 1 above, the poor diet group is dominated by the Proteobacteria phylum with a proportion of 46.3%. Next is Bacteroidetes with a proportion of approximately 23.8%. Firmicutes with a proportion of 17.5%. Actinobacteria was only found in two samples with a small proportion of approximately 3%. The F/B ratio in this group is 0.73. Meanwhile, the group with food quality needing improvement shows a more diverse and balanced microbiota composition, dominated by Firmicutes with a proportion of 50%, followed by Bacteroidetes with a proportion of approximately 39.3%. Proteobacteria accounted for 8%, and Actinobacteria accounted for a small proportion of approximately 1.4%. The F/B ratio in this group was 1.27

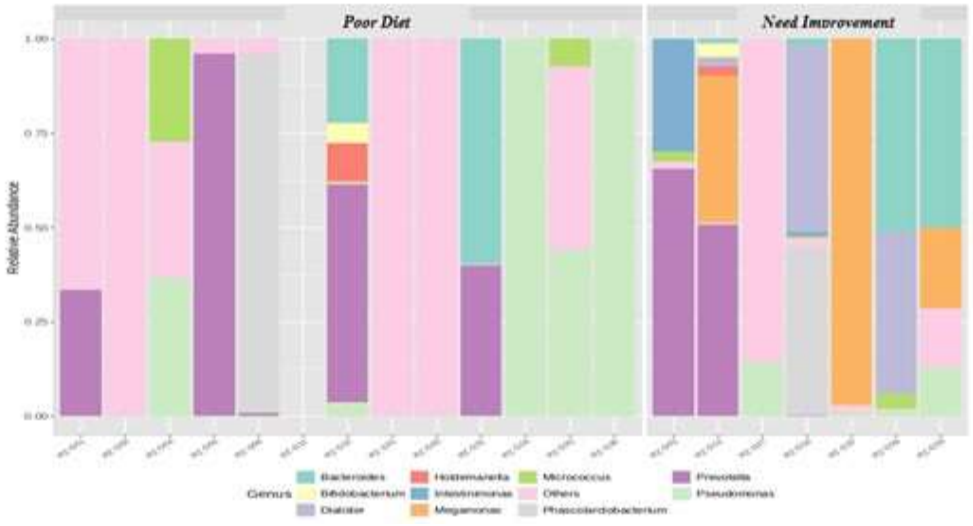

Figure 2. Comparison of Microbiota Profiles of Pregnant Women's Diet Quality at the Genus Level

As shown in Figure 2 above, the poor diet group is dominated by the genus Pseudomonas with a proportion of 21.5%. The genus Provotella with a proportion of 17.4%. The genus Phascolartobacterium with a proportion of 7.3%. Other genera are present but in relatively small quantities. In the 'need improvement' dietary quality group, the microbiota composition is more diverse and balanced, with dominance by Megamonas at 22%, Provotella at 16.4%, Bacteroides at 14.4%, and Dialister at 13.2%.

Correlation Between Diet Quality and Microbiota Profile

Based on the study conducted, the correlation between diet quality and microbiota profile is shown in Figure 3 below:

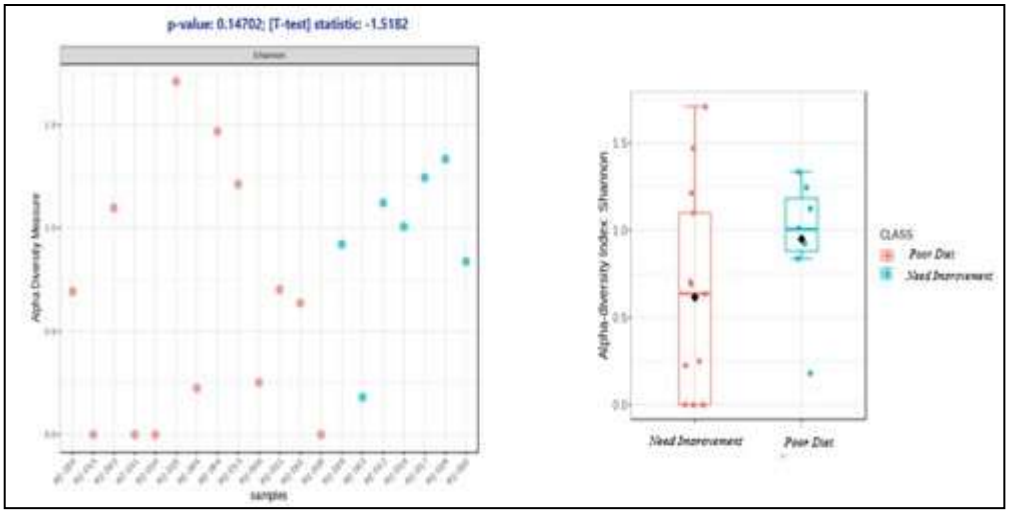

Figure 3. Alpha Diversity Microbiota Profile

Statistical test results showed a p-value of 0.14702 and a t-statistic value of -1.5182, indicating that there was no statistically significant difference between the group with food quality needing improvement and the poor diet group ($p > 0.05$).

DISCUSSION

An adequate nutrient intake is essential for pregnant women, as these nutrients are necessary for the mother to provide proper nutrition to the foetus for its growth and development in the womb. Foetal growth and birth weight are influenced by the nutrients consumed by the mother during pregnancy. Adequate nutrient intake in pregnant women can prevent malnutrition in the mother, which, if left unaddressed, can negatively impact foetal development, leading to low or excessive birth weight.

Diet quality is a parameter that reflects an individual's dietary intake. Diet quality can be measured based on adherence to balanced nutrition guidelines or health recommendations, such as preventing chronic diseases like anaemia. A good diet quality is expected to prevent anaemia in pregnant women. A study by Davidson et al. (2022) reported that most pregnant women have energy and protein sufficiency levels in the severe deficiency category. Meanwhile, vitamin C sufficiency levels in the adequate and insufficient categories are relatively balanced(8).

The Firmicutes to Bacteroidetes ratio (F/B) is one of the most commonly used indicators to describe microbiota balance. Balance between the two is crucial in regulating energy metabolism, inflammation, and immune homeostasis. A high F/B ratio, exceeding 2, has been associated with risks of obesity, insulin resistance, and chronic inflammation. Conversely, a low ratio is often linked to malnutrition, inflammatory bowel disease, and poor nutritional status(9).

Based on research findings, the Firmicutes/Bacteroidetes ratio (F/B ratio) is 1.06. A study by Magne et al. (2020) states that an F/B ratio approaching 1 indicates the absence of extreme dominance by a particular phylum and signifies a balanced microbiota. Pregnant women with poor diet quality tend to have a higher F/B ratio and less diverse microbiota profiles, especially in the second trimester (10). Research by Koren et al. (2012) explains that the microbiota

composition in the second and third trimesters experiences a significant increase in Firmicutes to support the foetus's energy needs and the mother's hormonal changes(11).

Dietary quality is the primary determinant of F/B ratio variation. A high-fibre diet, such as vegetables and fruits, increases the population of Bacteroidetes because this group is capable of degrading complex polysaccharides. Conversely, a diet high in saturated fat and low in fibre increases Firmicutes, resulting in a higher F/B ratio(12). Other factors influencing the F/B ratio include gestational age, hormonal status, and the mother's immune system. Progesterone and estrogen levels increase significantly during pregnancy and are known to modulate gene expression in microbes as well as the mother's immune response to commensal microbes. These hormonal changes tend to shift the microbiota composition toward an increase in Firmicutes, but this shift is more pronounced in the third trimester(13)

The p-value in the analysis results did not show statistical significance, which may be due to factors such as sample size limitations or biological variability among individuals. A study by Arrieta et al. (2015) states that in populations with small sample sizes, changes in microbiota diversity may not yet show statistical significance but remain biologically important (14). Blood microbiota falls under the category of 'low-biomass' microbiota, which is significantly less abundant compared to gut microbiota. According to Castillo et al. (2019), due to the sterile nature of blood, changes in microbial composition within it are not easily detected or accurately measured without the influence of infection or other pathological conditions (2). It can caused the relationship between dietary intake and blood microbiota profiles are not significant. Additionally, non-significant results may also be influenced by genetic, metabolic, and other health conditions. Rothschild et al. (2018) emphasise that microbiota variation is more influenced by non-dietary factors, such as genetic factors, medications (antibiotics), and an individual's inflammatory and immunological status(15).

The results show a dominance of Proteobacteria in poor diet group. Several studies have linked the relative increase in Proteobacteria to systemic inflammatory conditions and microbial dysbiosis. The low proportion of Firmicutes in the group with poor diet quality may be associated with inadequate nutrient intake, particularly fibre, protein, and micronutrients. Firmicutes are known to play a significant role in fermenting dietary fibre into short-chain fatty acids (SCFAs) such as butyrate, acetate, and propionate, which provide anti-inflammatory benefits, maintain intestinal mucosal integrity, and support energy metabolism(16). A study by David et al. (2014) showed that a diet rich in fibre, fruits, and vegetables contributes to an increase in the proportion of Firmicutes in the microbiota(17).

The results showed a dominance of the Bacteroidetes phylum in good diet group, which has been associated with a high-fibre, balanced, and low-saturated-fat diet. In the context of pregnancy, the presence of Bacteroidetes in a dominant proportion indicates adequate intake of plant-based foods, including vegetables, fruits, and whole grains, which provide complex polysaccharides as the primary fermentation substrate for these bacteria(18). Bacteroidetes plays a role in the fermentation of complex carbohydrates and the production of short-chain fatty acids (SCFAs), which are important for mineral absorption, including

iron. SCFAs such as butyrate also help maintain intestinal mucosal integrity, which can facilitate nutrient absorption (19). The main genera in this phylum, such as Bacteroides and Prevotella, are known to be involved in the degradation of complex carbohydrates and the production of short-chain fatty acids (SCFAs) such as acetate and propionate. SCFAs have systemic effects in enhancing intestinal mucosal integrity, suppressing inflammation, and improving iron absorption. Propionate produced by Bacteroidetes has metabolic benefits such as inhibiting cholesterol synthesis and modulating insulin sensitivity, as well as supporting balanced hepcidin synthesis. Controlled hepcidin ensures optimal iron absorption in the intestine(20).

CONCLUSION

This study provides information on the relationship between the quality of food consumed by pregnant women and the composition of microbiota in the blood. Although the statistical test results did not show a significant numerical relationship ($p > 0.05$), the data indicate that pregnant women with better eating habits tend to have a more balanced blood microbiota composition. This study also supports the use of blood microbiota as a potential systemic indicator for assessing the nutritional and immune status of pregnant women, particularly in developing countries where microbiota research is still limited.

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