Vol 8 No 2, July 2024

ANDALAS OBSTETRICS AND GYNECOLOGY JOURNAL

Correspondence Address:
Editorial Room Andalas Obstetrics and Gynecology Journal, 2nd Floor Obstetrics and Gynecology Department. M. Djamil Central General Hospital Padang, Jl. Perintis Kemerdekaan Padang, West Sumatra 25127
Website:
http://jurnalobgin.fk.unand.ac.id/

eISSN : 2579-8324 pISSN : 2579-8413

RESEARCH ARTICLE

Comparison of *Lactobacillus Plantarum*, *Lactobacillus Mucosae* and *Lactobacillus Farciminis* Bacteria Between Polycystic Ovary Syndrome and Non Polycystic Ovary Syndrome Students at Medical Faculty of Andalas University

Mentari Faisal Putri,[1,*] Haviz Yuad,[2] Andani Eka Putra[3]

[1] *Department of Obstetrics and Gynecology, Faculty of Medicine, Andalas University/Dr. M. Djamil Hospital, Padang, West Sumatra, Indonesia*
[2] *Endocrinology and Reproductive Fertility Division, Department of Obstetrics and Gynecology, Faculty of Medicine, Andalas University/Dr. M. Djamil Hospital, Padang, West Sumatra, Indonesia*
[3] *Center for Infectious Disease Diagnostics and Research, Faculty of Medicine, Andalas University/Dr. M. Djamil Hospital, Padang, West Sumatra, Indonesia*

Correspondence: *Mentari Faisal Putri; mentari.faisalputri@gmail.com ; +62 812-8548-3420*

DOI: *https://doi.org/10.25077/aoj.8.2.732-745.2024*

Abstract

Background*: Polycystic Ovary Syndrome (PCOS) is a disorder characterized by hyperandrogenism, ovulatory dysfunction, and morphological features of polycystic ovaries. It is associated with insulin resistance and hyperinsulinemia. Gut microbial dysbiosis plays a role in PCOS, which is associated with insulin resistance, hyperandrogenism, chronic inflammation, and metabolic syndrome. PCOS treatment with Probiotics maintains the stability and diversity of the gut microbiome.*
Objective*: To determine the comparison of Lactobacillus plantarum, Lactobacillus mucosae, and Lactobacillus farciminis Bacteria Between Polycystic Ovary Syndrome and Non Polycystic Ovary Syndrome Students at the Medical Faculty of Andalas University.*
Method*: This research was an analytic observational study using a cross-sectional method conducted on female students with Polycystic Ovary Syndrome and Non-Polycystic Ovary Syndrome at the Medical Faculty of Andalas University.*
Results: *Bivariate analysis of microbiota comparison of female students with PCOS and without PCOS, Mann-Whitney Test on L.mucosae (p=0.774), while Independent T-Test analysis on L.farciminis (p=0.006).*
Conclusion*: There is a decrease in microbiota L. Mucosae in female students with PCOS, but there is no significant difference. There is a significant comparison in the number of microbiota L.farciminis in female students with and without PCOS at FK Unand.*

Keywords: *Polycystic Ovary Syndrome, gut microbiota.*

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https://doi.org/10.25077/aoj.8.2.732-745.2024

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a disorder characterized by hyperandrogenism, ovulatory dysfunction, and morphological features of polycystic ovaries. World Health Organization (WHO) data shows that approximately 116 million women (3.4%) are affected by PCOS globally. Manifestations of androgen excess (e.g., hirsutism) can cause substantial abnormalities in patients, and polycystic ovary syndrome is the most common cause of anovulatory infertility.[1] Women with PCOS usually have infrequent and irregular periods. Amenorrhea with symptoms of hyperandrogenism (acne, excess facial and body hair, or thinning of scalp hair) is highly suggestive of PCOS.26 Women with endocrine disorders also have higher rates of obesity, dyslipidemia, and insulin resistance. PCOS is associated with insulin resistance and hyperinsulinemia. Obesity and increased waist-hip ratio are commonly found in women with PCOS.[2,3]

Recent studies have shown that the gut microbiota plays a role in PCOS. Altered microbiota composition (dysbiosis) associated with insulin resistance, hyperandrogenism, chronic inflammation, and metabolic syndrome influences the clinical manifestations of PCOS through short-chain fatty acids, lipopolysaccharides, sex hormones, and the brain-gut axis. With the increasing understanding of gut microbiota in the pathogenesis of PCOS, the use of microbiota-targeted agents (such as probiotics, prebiotics, and synbiotics) in treating PCOS has been discussed recently.[4]

The gut microbiota is an "endocrine organ" that maintains human health and affects the reproductive endocrine system by interacting with estrogens, androgens, insulin, and others. Disorders of gut microbiota are involved in endotoxemia, short-chain fatty acid production, bile acid metabolism, and abnormal secretion of intestinal brain peptides. The above physiological and pathological processes are related to PCOS manifestations such as hyperandrogenism, insulin resistance, chronic inflammatory reactions, and abnormal levels of gut-brain peptides. Therefore, gut microbiota may affect follicular development, sex hormones, and metabolic rate through hyperandrogenism, insulin resistance, chronic inflammation, and brain-gut axis and participate in the pathogenesis of PCOS.[5]

Individual genetic and environmental factors influence the gut microbiome's dynamic and variable microbial community. Sequencing methods have enabled advances in studies on the gut microbiome. Knowledge of microbiota composition and bacterial gene activity has increased. Probiotic microorganisms are a new perspective of gut microbiota modulation. Lactic acid bacteria (LAB) comprise an important source of probiotics in food products. Due to their beneficial properties, including maintenance of the gut microbiota and disease prevention, LAB is becoming more frequent.[6] Lactic acid bacteria are referred to as part of the good bacteria. They belong to the normal gut flora and are beneficial for human health. Lactic acid bacteria, particularly rod-shaped lactobacilli, are generally regarded as friendly disposition. They are members of the normal microbial ecosystem in the intestinal tract and play a key role in maintaining the stability and diversity of the gut microbiome.[7]

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https://doi.org/10.25077/aoj.8.2.732-745.2024

METHODS

This research was conducted at the Faculty of Medicine, Andalas University, from February to June 2023. The population in this study were all students of the Faculty of Medicine, Andalas University. The sample for this study were all Faculty of Medicine, Andalas University students who met the inclusion criteria, and there were no exclusion criteria. The sample size was determined using the formula for the difference in the means of two independent populations. From the above equation, the minimum number of research subjects in both groups is 19 people.

Stool sampling

Trained enumerators assisted in stool sample collection. Subjects were instructed to contact the enumerator if they were about to defecate. The subjects visited were subjects who contacted the enumerator and defecated around 07.00-15.00 WIB. Feces were put into a sterile tube provided and should not be mixed with urine, toilet water, or other impurities. Feces that are inserted are about 2-5g. The sterile tube was labeled with the code, respondent's name, date, and time of collection. Sterile containers containing stool samples were brought in a coolbox containing ice gel and immediately analyzed at the Microbiology Laboratory of FK Unand.

DNA isolation

Freshly obtained fecal samples were stored at -20°C until the time of use. Then, weigh 200 mg of the stool sample to be analyzed. Suspend in 9 ml Buffer C, then vortex for 20 minutes. Place in a 10 ml injection syringe and filter using a >0.75 µm filter or sterile cloth. The liquid is centrifuged at 12,000 rpm for 10 min. The pellet is washed 3 times with Buffer C (1000 µl/1 ml), vortexed, and then centrifuged at 60 rpm for 5 minutes. The pellet was suspended with 200 µl lysozyme digestion buffer and vortexed. The procedure was continued according to Promega protocol.

Serial Dilution

Serial dilution is used to make a standard curve, where bacterial culture is done first, followed by suspension with 2 ml PBS, comparison with McFarland scale 1 (3 x 108), DNA isolation, and real-time PCR.

Standard Curve

The standard curve was prepared based on the number of target lactic acid bacteria that were serially diluted. DNA isolation was performed at each dilution stage, and real-time PCR was conducted for each DNA group. The Cq value obtained will be compared with the concentration of bacteria. At the end of this stage, a standard curve will be obtained that can be used to measure the number of bacteria.

Real-Time PCR

Real-Time PCR (Bioline Kit) for its Master Mix composition consists of 10 µl 2 x SensiFAST Probe No-ROX Mix, 0.8 µl Foward Primer, 0.8 µl Reverse Primer, 0.2 µl Probe, 5 µl Sample, and 3.2 µl H2O. The real-time PCR cycle consisted of 1 cycle of Polymerase Activation at 95°C for 2-5 minutes, 40 cycles (Denaturation at 95°C for 10 seconds, Anealling/Extension at 60°C for 20-50

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seconds), using the CFX 96 real-time PCR system (Biorad, USA).

RESULT AND DISCUSSION

Table 1. Characteristics of Research Respondents

Characteristics	PCOS (n=21)	Non-PCOS (n=20)
BMI		
BMI (kg/m^2), mean± SD	24,57 ± 6,045	21,55 ± 2,103
Max (kg/m^2)	42,06	26,67
Min (kg/m^2)	17,54	18,29
Obesity		
Not Obesity (<25 kg/m^2)	14 (66,7%)	19 (95,0%)
Obesity (≥ 25 kg/m^2)	7 (33,3%)	1 (5,0%)
Clinical hyperandrogenismism	21 (100%)	5 (25,0%)
Acanthosis nigricans	6 (28,6%)	1 (5,0%)

Based on Table 1, the mean BMI in PCOS students is 24.57 Kg/m^2 with a standard deviation of 6.045; the highest BMI in PCOS students is 42.06 Kg/m^2, while the lowest is 17.54 Kg/m^2. The mean BMI in female students without PCOS was 21.54 Kg/m^2 with a standard deviation of 2.103. The highest BMI in female students without PCOS was 26.67 Kg/m^2, while the lowest was 18.28 Kg/m^2. In female students with PCOS, as many as 7 people (33.3%) were obese, and 14 (66.7%) people were not obese, while there were female students without PCOS obtained as many as 1 person (5.0%) were obese, and 19 people (95.0%) were not obese.

The number of female students with PCOS with clinical hyperandrogenism is 21 people (100%), while the number of female students without PCOS with clinical hyperandrogenism is 5 people (25%). The number of female students with PCOS with acanthosis nigricans is 6 people (28.6%), while the number of female students without PCOS with acanthosis nigricans is 1 person (5.0%).

Table 2. Mean Intestinal Microbiota in PCOS and non-PCOS College Students

Microbiota	Variable	N	Mean (Log Cfu/ mL) ±SD	Max (Log Cfu/mL)
Labtobacillus mucosae	PCOS	21	5,66±0,59	7.44
Lactobacillus farciminis	PCOS	21	6,56±0,50	7,48
Labtobacillus mucosae	Not PCOS	20	5,65±0,38	6,43
Lactobacillus farciminis	Not PCOS	20	6,08±0,56	7,24

Based on Table 2, the average *Lactobacillus mucosae* is 5.66 ± 0.58 Log Cfu/ml, with the highest number in PCOS female students being 7.44 Log Cfu/ml, the average Lactobacillus facilities is 6.56 ± 0.50 Log Cfu/ml, with the highest number in PCOS students being 7.48 Log Cfu/ml. The mean *Lactobacillus mucosae* was 5.65±0.38 Log Cfu/ml, with the highest number in female students without PCOS being 6.43 Log Cfu/mL, the mean *Lactobacillus farciminis* was 6.08±0.56 Log Cfu/ml, with the highest in female students without PCOS it was 7.24 Log Cfu/mL.

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Table 3. Comparison of the number of intestinal microbiota Lactobacillus mucosae and between PCOS and non-PCOS college students

Variable	Subject		Total n (%)	p-value
	PCOS (n=21)	Tidak PCOS (n=20)		
*Lactobacillus mucosae**	5,43	5,73	41 (100%)	0,419
*Lactobacillus farciminis***	6,56±0,50	6,08±0,56	41 (100%)	0,006

*Mann-Whitney Test
**Independent T-Test

The normality test used in this study was the Shapiro-Wilk test because the amount of data in this study was <50 data. The sapphire-will test normality test showed that the data of the variable number of gut microbiota had a normal distribution for Lactobacillus facilities bacteria in female students with and without PCOS with a p-value of 0.089 but an abnormal distribution for *Lactobacillus mucosae* bacteria in female students with PCOS with a p-value of 0.005. The results of the Shapiro-will test normality test became the basis for using the Mann-Whitney Test / Independent T-test bivariate analysis to determine the comparison of the number of intestinal mycobacteria in PCOS and non-PCOS female students. Normally distributed data was conducted by the Independent T-test, while non-normally distributed data was conducted by the Mann-Whitney Test. Independent T-Test bivariate analysis gave a p=0.006 value for *Lactobacillus farciminis*. This shows a significant comparison in this study's number of *Lactobacillus farciminis* of PCOS and non-PCOS female students (Table 3).

Table 4. Relationship of Menstrual Cycle Disorder, Clinical Hyperandrogenism, Obesity, and Acanthosis nigricans with the number of mucosal Lactobacillus

Variable	*Lactobacillus mucosae* (Median)	p-value
Menstrual cycle		
Normal	5,73	0,419
Disrupted	5,43	
Clinical Hyperandrogenism		
Yes	5,42	0,076
No	5,76	
Obesity		
Yes	5,43	0,339
No	5,71	
Acanthosis nigricans		
Yes	5,44	0,623
No	5,68	

Based on Table 4, the study results are displayed as medians because the data distribution is abnormal, so they are not displayed as averages. Mann-Whitney Test bivariate analysis showed p values for the relationship of menstrual cycle disorders (p=0.419), clinical hyperandrogenism (p=0.076), obesity (p=0.339), and acanthosis nigricans (p=0.623) with the number of *Lactobacillus mucosae*. This shows that there is no significant relationship between menstrual cycle disorders, clinical hyperandrogenism, obesity, and acanthosis nigricans with the number of *Lactobacillus*

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mucosae in this study.

Table 5. Relationship between menstrual cycle disorders, clinical hyperandrogenism, obesity, acanthosis nigricans with the number of Lactobacillus farciminis

Variable	*Lactobacillus farciminis*	*p-value*
Menstrual cycle		
Normal	6,08±0,56	0,006
Disrupted	6,56 ±0,50	
Clinical Hyperandrogenism		
Yes	6,50±0,52	0,007
No	6,01±0,56	
Obesity		
Yes	6,64 ±0,50	0,086
No	6,25±0,57	
Acanthosis nigricans		
Yes	6,56 ±0,49	0,236
No	6,27±0,59	

Based on Table 5, the study results are displayed in the form of averages because the distribution of *Lactobacillus farciminis* data is normally distributed so that an independent t-test can be performed. Bivariate analysis of the independent t-test showed the p-value for the relationship between menstrual cycle disorders (p=0.006) and clinical hyperandrogenism (p=0.007) with the number of *Lactobacillus farciminis*. This indicates that there is a significant relationship between menstrual cycle disorders and clinical hyperandrogenism with the number of *Lactobacillus farciminis* in this study. Bivariate independent t-test analysis showed p values for the association of obesity (p=0.086) and acanthosis nigricans (p=0.236) with the number of *Lactobacillus farciminis*. This indicates no significant relationship between obesity and acanthosis nigricans and the number of *Lactobacillus farciminis* in this

CONCLUSION

Based on the results of the research Comparison of *Lactobacillus plantarum*, *Lactobacillus mucosae*, and *Lactobacillus farciminis* Bacteria Between Polycystic Ovary Syndrome and Not Polycystic Ovary Syndrome Students at FK Unand, it can be concluded that: characteristics of female students with PCOS as many as 33.3% of students are obese and 28.6% of students have acanthosis nigricans. The mean intestinal microbiota of *Lactobacillus mucosae* was 5.66 ± 0.59 Log Cfu/mL. The mean of *Lactobacillus farciminis* was 6.56 ± 0.50 Log Cfu/mL in female students with PCOS, while the mean intestinal microbiota of *Lactobacillus mucosae* was 5.65 ± 0.38 Log Cfu/mL, and the mean of *Lactobacillus farciminis* was 6.08 ± 0.56 Log Cfu/mL in female students without PCOS at the Faculty of Medicine.

There was a significant difference between the mean number of *Lactobacillus farciminis* in female students with PCOS and female students without PCOS, and there was no significant difference between the mean number of *Lactobacillus mucosae* in female students with PCOS and female students without PCOS at FK Unand. There is a significant relationship between menstrual cycle disorders and hyperandrogenism with the number of Lactobacillus farciminis, and there is no

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https://doi.org/10.25077/aoj.8.2.732-745.2024

significant relationship between menstrual cycle disorders, hyperandrogenism, obesity, acanthosis nigricans with the number of *Lactobacillus mucosae* in female students at FK Unand.

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