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Original Research Article

A Comparative Evaluates and Analyze the Microbiomes Component of the Vagina in Females with Polycystic Ovarian Syndrome (PCOS)

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Abstract

Aim: The aim of the present study was to evaluate and analyze the microbiomes component of the vagina in females with polycystic ovarian syndrome PCOS, and compare it with that of healthy females.

Methods: The present study was conducted in the Department of Obstetrics and Gynaecology, Darbhanga Medical College and Hospital, Darbhanga, Bihar, India for 1 year. A case-control study in which the microorganisms settling in the vaginal area was compared for two groups of women, the first group suffering from PCOS and the second group being the control group. The study included 100 females.

Results: We compared the sociodemographic data, the mean age of PCOS patient is (27.3±951) while mean age of control group is (29.1±0.786), (4%) were smokers in PCOS group and (2%) in controls, the PCOS patient tends to be obese (mean of BMI is 27.3±4.26) while the mean of BMI for control group is (22.8±2.12). We found that there is significant difference in mean of the frequency of menstrual cycle between both study groups (5.6±1.7) and (11.5±1.6) respectively (p- value < 0.0001, 45 cases of total 50 PCOS cases were have oligomenorrhea while 1 out of 50 control group have oligomenorrhea. All PCOS cases had Ultrasound feature of polycystic ovaries while control group have no case have this feature; for biochemical and clinical feature of hyperandrogenism, p-value was significant between both study groups.

Conclusion: There is large diversity in the vaginal microbiota with disruption to normal flora in PCOS affected patients so we need further studies to evaluate the relationship between the microbiota.

Keywords: Poly cystic ovarian syndrome (PCOS), Vaginal microbiota, gut microbiomes, mechanism, therapeutics

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common causes of infertility with ~10% global prevalence. [1] Due to the unclear etiology of PCOS, it is necessary to explore the potential influencing factors to develop effective therapeutic strategies. [2] The most common clinical symptoms include hyperandrogenism, oligomenorrhea, and polycystic ovarian morphology, often accompanied by obesity and acne. [3] Clinically, different treatments are recommended for various symptoms. Therefore, efforts to develop precise prevention and intervention approaches should take into account the complexity of PCOS mechanisms.

It was reported that the prevalence of PCOS in premenopausal women is up to almost 20% as the current, more inclusive definitions [4–6], which suggesting PCOS the most common endocrine and metabolic disorder in women at reproductive age. As the increasing studies aiming at the microbiota, it is widely believed that microbiota has evolved together with the hosts and is becoming an integral part of the human body. [7] It is well acknowledged that the microbiota is responsible for more than 95% of the genetic activity of the organism. [8] The microbiota was called as "second genome" for the human body, indicating that microbiota exerts vital function in human health. [9] Various kinds of microbiota are acknowledged to play roles in influencing physiology balance [10], metabolism process [11], nutrition production [12], and immune mediation [13] under physiological conditions. The complex and delicate balance between the microbiota and the host maintain the health of human.

The most widely accepted hypothesis is that PCOS is a genetically determined syndrome, but with clinical and biochemical variability which is determined by the combination of genetic and environmental influences. [14] Menstrual irregularity hyperandrogenism, and polycystic ovary morphology are some of the parameters used to diagnose PCOS (PCOM). According to NIH 2012/International PCOS Guidelines 2018, the involvement of at least two of the following three criteria must be present in order to diagnose PCOS: oligo- and/or anovulation, hyperandrogenism (clinical or biological), and polycystic ovaries. [15] PCOS has a significant influence on subsequent women's health because it triggers several metabolic disorders such as insulin resistance (IR), diabetes, and obesity. [16]

The aim of the present study was to evaluate and analyze the microbiomes component of the vagina in females with polycystic ovarian syndrome PCOS, and compare it with that of healthy females.

Methods

The present study was conducted in the Department of obstetrics and Gynaecology, Darbhanga Medical College and Hospital, Darbhanga, Bihar, India for 1 year. A case-control study in which the microorganisms settling in the vaginal area was compared for two groups of women, the first group suffering from PCOS and the second group being the control group. The study included 100 females.

Data that were collected included demographic data, detailed information's about signs and symptoms of PCOS, full medical, surgical, family, social and drug history.

Study population and exposure variable

All women in both groups of reproductive age, divided into 2 groups first group includes PCOS patient and the second group includes women without PCOS presents to outpatient clinics for other complains like vaginal discharge, the

Rotterdam Criteria were used to diagnose PCOS,

Diagnosis of PCOS, requiring two of three features: anovulation or oligo-ovulation (irregular menstrual periods (oligomenorrhoea or amenorrhea)), clinical and/or biochemical hyperandrogenism, and polycystic ovaries by ultrasound. [17]

Informed consent was obtained from each participant. The significant and purpose of the study was explained to women. Confidentiality of any obtained information was ensured.

Exclusion criteria

For both groups, all patients with endocrinal abnormalities like thyroid disorder, diabetes, hyperprolactinemia, Cushing's syndrome and cancer were excluded. Pregnant, lactating and menstruating women were also excluded.

All patients any antibiotics oral or vaginal for the last 7 days before the test, no hormonal treatment and no sexual relationship within 48 hours.

For the control group, healthy women whose ages were similar age as the PCOS patients were selected. They visit outpatient clinic for fertility problems or contraceptive purposes. All their physical examination indexes were normal.

Statistical design

Data was collected, coded, tabulated and analyzed, using the SPSS computer application for statistical analysis. Descriptive statistics was used to calculate percentages and frequencies.

Sample collection

Collection of swabs was done under complete aseptic technique, high vaginal swabs HVS were gathered at the day of patient visit for outpatient clinic. After opening of swab package, the swab shaft was hold in the middle without touching the tip of the swab, then it inserted about 5-7 cm in the vagina carefully and rotate for 20-30 seconds, after the swab absorbed the moisture from vaginal wall, it withholds without touching the skin of the perineum. Vaginal swabs were immediately placed in a clean tube containing 0.5 ml sterile saline. Samples were placed immediately in a refrigerator or ice bucket at 4- 8◦C and then at -20◦C in less than 4 hours. All the instruments used in these steps were sterilized.

Laboratory methods

The Vitek 2 Compact (30 card capacity) system can identify organism by utilizing a fluorogenic methodology also can test susceptibility by using a turbidimetric method depending on a 64 barcoded well card with information on card type, lot number and unique card identification number and expiration date. ID-GN (gram negative bacillus cation), ID-GP (gram positive cocci identification), AST-GN (gram negative susceptibility), and AST-GP (gram positive susceptibility) are some of the test kits available. Within 10 hours, the Vitek 2 ID-GN card can identify 154 Enterobacteriaceae species and a limited set of glucose non-fermenting gram negative organisms. Within 8 hours or fewer, the Vitek 2 ID-GP card can identify 124 staphylococci, streptococci, enterococci, and a limited set of gram-positive organisms. [18]

Results

Table 1: Demographic characteristic of study groups

Characteristic	PCOS (n= 50) N(%)/Mean ±SD	Control (n = 50) N(%)/Mean ±SD	Mean difference or odds ratio (95% CI)	p-value
Age	25.5±950	29.1±0.786	1.4446 to 2.1160	< 0.0001
Smoking	2(4%)	1(2%)	5.9157% to 9.8136%	0.5505
BMI	27.3±4.26	22.8±2.12	-5.8073 to --3.3927	< 0.0001

In Table 1 we compared the sociodemographic data, the mean age of PCOS patient is (27.3±951) while mean age of control group is (29.1±0.786), (4%) were smokers in PCOS group and (2%) in controls, the PCOS patient tends to be obese (mean of BMI is 27.3±4.26) while the mean of BMI for control group is (22.8±2.12).

Table 2: PCOS signs and symptoms of study groups

	PCOS (n= 50) N(%) / Mean ±SD	Control (n = 50) N(%) / Mean ±SD	Mean difference or odds ratio (95% CI)	p-value
Frequency of menstrual cycle/1 year	5.6±1.7	11.5±1.6	5.2393 to 6.3607	< 0.0001
Oligomenorrhea hyperandrogenism	45(90%)	1(1.66%)	84.2225% to 97.7779%	< 0.0001
Testosterone level (nmol/L)	2.8±0.6	1.4±0.5	-1.5997 to -1.2003	< 0.0001
Acne	20(40%)	4(8%)	17.1054% to 44.9489%	< 0.0001
Hirsutism	30(60%)	5(10%)	34.0734% to 62.5260%	< 0.0001
Alopecia	2(4%)	0	-3.1528% to 11.3590%	0.1560
Ultrasound feature of polycystic ovaries	50(100%)	0	91.4904% to 100.0000%	< 0.0001

In Table 2 sign and symptoms of PCOS and control group, we found that there is significant difference in mean of the frequency of menstrual cycle between both study groups (5.6±1.7) and (11.5±1.6) respectively (p- value < 0.0001, 45 cases of total 50 PCOS cases were have oligomenorrhea while 1 out of 50 control group have oligomenorrhea. All PCOS cases had Ultrasound feature of polycystic ovaries while control group have no case have this feature; for biochemical and clinical feature of hyperandrogenism, p-value was significant between both study groups.

Discussion

Polycystic ovary syndrome (the common abbreviation is “PCOS”) is widely acknowledged as an endocrine disorder that affects almost 10% of reproductive age women. PCOS is characterized by hyperandrogenism [19], ovarian dysfunction, and metabolic syndrome. The main symptoms included hirsutism, irregular menstrual periods, and ovarian cysts. As the deepening of the studies, PCOS is also regraded as a kind of metabolic disease, with the symptoms including increased triglycerides, low-density lipoprotein cholesterol and insulin resistance indices. [20] Since the amount of females disturbed by PCOS increases and the mechanism of PCOS is still unclear, there is an increasing studies conducting relevant experiments. Many studies have revealed the relevance of the relationship between the alteration of pathogenic factors [21] (including lifestyle, obesity, genetic factors and so on) and PCOS. However, few studies have explored the relationship between microbes and PCOS, especially the vaginal microbes and PCOS.

In our study we found that both groups of the study had abundant Lactobacillus species in their vagina as most of similar studies found that in the majority of women, those species are the most common vaginal bacteria. [22,23]

L.crispatus was found in all participants of both groups, but its concentration may be altered as many other microbiotas was detected in the HVS of the first group, those microbiotas was not detected in control group, L. jensonii and L. gasseri was detected in more frequent in control group than PCOS group, this result is similar to result of other study done by Xiang Hong et al. in 202019 and Yaoyao Tu and et al. 2020. [24]

Three potential pathways have been proposed to explain the association between the vaginal microbiome and female hormone levels. Initially, sex hormones affect mucosal immunity to determine the vaginal microbiome. [25] Antigen presentation, cytokine production, immunoglobulin production and transport are influenced by variations in sex hormones levels [26] and vaginal mucosal immunity directly impacts the stability of the vaginal microbiome. Meanwhile, estrogen stimulates accumulation of glycogen in the vaginal epithelium, which is thought to play a major role in maintaining protective Lactobacillus-dominated microbiota. [25] Secondly, disorder of the vaginal microbiome potentially leads to an increase in local inflammatory factors, such as interleukin-8 and tumor necrosis factor –a [27], which, along with a number of metabolites, trigger chronic systemic inflammation through the blood and lymph system, and in turn, affect the hypothalamic-pituitary-ovarian axis. [28]

In this study we did not detect Chlamydia trachomatis or Neisseria gonorrhoeae in both groups of the study, this result not agreed with Yaoyao and et al study who found abundant Chlamydia trachomatis in the PCOS group, this difference in the result may be explained by the cultural, social and ethnic diversity between both communities of the studies in spite of the closer number of sample size. [29]

Conclusion

There is large diversity in the vaginal microbiota with disruption to normal flora in PCOS affected patients so we need further studies to evaluate the relationship between the microbiota. The significant variations in vaginal bacterial populations were recorded among PCOS patients with distinct clinical manifestations, in particular, different testosterone levels. Further research is essential to establish the microbiotal factors underpinning this disease and provide new insights that could facilitate improvement of PCOS prevention, screening and treatment strategies.

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