A case-control study to evaluate dysbiosis in gut microbiome in Indian women with PCOS

Description of research to be carried out

Introduction: Polycystic ovary Syndrome (PCOS) is a complex, chronic endocrine disorder affecting 4% to 20% of women in the reproductive age group, globally. PCOS is the diagnosis of exclusion and lifestyle interventions including diet and nutrition are the mainstay of the management. The development of Insulin Resistance is considered to be a crucial step in its etiopathogenesis. However, Insulin resistance is not included as a diagnostic or prognostic marker for PCOS. Gut microbiota and their dysbiosis were found to play an important role in many metabolic diseases through various metabolites synthesized by the gut microbiome. These metabolites are further involved in the causation of Insulin Resistance, Hyperandrogenism, and Ovulatory dysfunction. It is hypothesized that dietary modifications improve clinical features of PCOS, and its phenotypes by correcting dysbiosis and hence reducing insulin resistance.

Rationale: Diet-nutrition and Gut microbiome: Diet and nutrition are known to play a critical role in the development of conditions like PCOS, Type 2 DM, and Insulin Resistance. Maintenance of optimum nutritional status is pivotal in the prevention and treatment of PCOS. Even though therapeutics are introduced, dietary habits play a significant role in the reversal of PCOS symptoms. However, there are no clear recommendations on the type and duration of diet to be advised to PCOS patients. PCOS, IR, and Gut. Studies in other countries have shown that dysbiosis of gut microbiota occurs in PCOS, where diversity decreases, and the abundance of some bacteria species is related to metabolic disorders. Some studies have shown a decrease in certain bacteria such as Faecalibacterium prausnitzii, Blauti, while some studies have shown these bacteria's abundance. Similarly, the PCOS-IR group has shown a relative abundance of Rothia, Ruminococcus, and Enterococcus was significantly higher and Prevotella was lower as compared to IR and control groups. The abundance of Enterococcus was positively correlated with waist circumference, hip circumference, diastolic blood pressure, and insulin resistance. Rothia abundance is positively associated with waist circumference and free fatty acids. On one hand, it is known that BCAAs play roles in anabolic effects on body weight, muscle prote and glucose homeostasis. On the other hand, some studies have also suggested that BCAAs are associated with insulin resistance, obesity, and even T2DM, which are similar to the phenotypes of PCOS. These results indicate that BCAAs may be involved in the onset of PCOS or serve as biomarkers for PCOS. Another mechanism is that some metabolites derived from the abnormal metabolism

may impair the function of islet β cell mitochondria. Through this study, it is proposed that dysbiosis of gut microbiota is an important pathogenic event in the development. Although the abnormalities in gut microbiota or their metabolites may not be the causal factors, they may have a significant role to play in the pathological process of PCOS. Microbiota-related metabolic pathways can significantly affect levels of inflammatory cytokines, insulin signaling, glucose metabolism, lipid metabolism, and hormonal imbalance in patients with PCOS. Hence the study hypothesizes that dysbiosis of gut microbiota and hence the disturbances in gut metabolites trigger the development of IR of variable intensity that can affect the development of PCOS and correction in the dysbiosis of the gut microbiome will possibly correct the IR and affects the progression or the regression of symptoms.

Research question

1. Is the dysbiosis of the gut or the alteration of the gut microbiome associated with PCOS
2. Dietary modifications result not only in the correction of dysbiosis associated with PCOS but also in the correction of altered metabolites responsible for insulin resistance and hyperandrogenism in patients with PCOS

Participants: Adolescent girls and women in early reproductive age Intervention: Counseling of therapeutic dietary modifications

Intervention: Counselling about dietary modification

Comparison: Adolescent girls and women in early reproductive age without PCOS

Outcome: Correction of dysbiosis and metabolites associated with the development of PCOS

Objectives:

Primary:

● To evaluate the microbial association from dysbiosis of the Gut microbiome in patients with PCOS in Indian women through clinical and biochemical markers and hence to study the differential microbiome in different phenotypes and the role of dietary changes.

Secondary:

● To understand the role of gut microbiota and their metabolites in patients with Insulin Resistance, Obesity, and hyperandrogenism in patients with PCOS.

● To study the impact of intervention in the form of dietary counseling on the correction of dysbiosis and hence in the progression or the regression of the clinical features of PCOS.

Brief description of pilot data, if available: Not available

Methodology

A hospital-based, case-control study will be conducted after IEC approval in participants fulfilling Inclusion-Exclusion criteria. Participants will be categorized into four groups based

on the presence and absence of PCOS and Obesity. Baseline data including clinical and anthropometric data, blood samples, and fecal samples will be collected. Participants will be counseled about dietary modification and followed up for 6 months. Endline data, blood samples, and fecal samples will be collected to evaluate the impact of the dietary interventions.

Study Design: An Analytical Observational Case-Control study followed by a Quasi-Experimental with an intervention in the form of dietary counseling.

Study duration: 24 months

Study sites: Bharati Hospital Research Centre, Pune, Gupte Hospital Pune

Sample Size: Considering interindividual microbiome variability, each sub-group will have 48 participants and thus in total 192 PCOS and 192 non-PCOS participants.

Study participants: Study participants will be randomly selected from the hospital settings. As the time interval between the occurrence of dysbiosis and the presence of a clinical picture of PCOS is unclear, we aim to enroll participants from a wide range of age groups. In adolescents, pubertal changes may mimic the features of PCOS, to differentiate them from pubertal changes they will be enrolled after 5 years of menarche.

Anticipated outcome: The study will evaluate the interplay between metabolic, inflammatory, and hormonal markers in patients with PCOS and its possible association with the dysbiosis of gut microbiome in Indian women based on different phenotypes of PCOS.

Timelines: GANTT Chart

Phases	Quarters → Activities ↓	1st	2nd	3rd	4th	5th	6th	7th	8th
Preparatory phase	IEC approval								
	Purchasing consumables								
	Development of SOPs								
	Staff recruitment								
	Training of the staff								
Data collection phase	Baseline data collection								
	Shipment of samples								
	Endline data collection								
Analysis and report writing	Laboratory analysis of samples								
	Electronic data entry with QC								
	Statistical analysis of the data								
	Report writing								

Bibliography

1. Liyun Duan, Xuedong An, Yuehong Zhang, De Jin, Shenghui Zhao, Rongrong Zhou et al. Gut microbiota as the critical correlation of polycystic ovary syndrome and type 2 diabetes mellitus. Biomedicine & Pharmacotherapy 142 (2021) 112094.
2. Che X.a,b · Chen Z.c · Liu M.b · Mo Z. Dietary Interventions: A Promising Treatment for Polycystic Ovary Syndrome. Ann Nutr Metab 2021;77:313–323.
3. Valentina Rodriguez Paris, Xin Yi Denise Wong, Samantha M Solon[1]Biet, Melissa C Edwards, Ali Aflatounian, Robert B Gilchrist, Stephen J Simpson, David J Handelsman, Nadeem O Kaakoush & Kirsty A Walters (2022) The interplay between PCOS pathology and diet on gut microbiota in a mouse model, Gut Microbes, 14:1, 2085961,
4. Nicole G Barra, Fernando F Anhê, Joseph F Cavallari, Anita M Singh, Darryl Y Chan and Jonathan D Schertzer. Micronutrients impact the gut microbiota and blood glucose Journal of Endocrinology (2021) 250, R1–R21.
5. Gamze Yurtdaş, and Yasemin Akdevelioglu. New Approach to Polycystic Ovary Syndrome: The Gut Microbiota. JOURNAL OF THE AMERICAN COLLEGE OF NUTRITION https://doi.org/10.1080/07315724.2019.1657515
6. Fangfang He and Yumei Li The gut microbial composition in polycystic ovary syndrome with insulin resistance: findings from a normal-weight population. Journal of Ovarian Research (2021) 14:50,
7. Jiayu Wu1,2,3, Kai Wang1,2,3, Xuemei Wang1,2,3, Yanli Pang Changtao Jiang. The role of the gut microbiome and its metabolites in metabolic diseases Protein Cell 2021, 12(5):360–373.
8. Zhang M, Hu R, Huang Y, Zhou F, Li F, Liu Z, Geng Y, Dong H, Ma W, Song K and Song Y (2022) Present and Future: Crosstalks Between Polycystic Ovary Syndrome and Gut Metabolites Relating to Gut Microbiota. Front. Endocrinol. 13:933110.