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ORIGINAL PAPER

Salivary microbial diversity – an investigation on possible biomarkers for polycystic ovarian syndrome from eastern India

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ABSTRACT

Introduction and aim. Polycystic ovarian syndrome (PCOS) is one of the major reproductive health issues, thought to be multifactorial, needs serious attention as a dual burden (health and economic) mainly for developing countries like India, due to its rapid rise (30%) in the last couple of years. Therefore, widespread and liberal screening for this disorder towards prognosis, diagnosis and intervention seems to be an urgent area of research. In this background, the present study attempts to unravel the association of salivary microbial diversity and PCOS.

Material and methods. To achieve the purpose 100 clinically diagnosed PCOS individuals and 110 age matched non-PCOS participants from Bengalee Hindu caste population, West Bengal, India was considered. Obtained salivary samples were identified with 16S rDNA amplification and microbial diversity were determined by Alu I restriction enzyme digestion.

Results. The present study revealed an explicit pattern of DNA fragment lengths varied between 200 bp and 225 bp in PCOs in comparison to the non-PCOS group.

Conclusion. The cardinal feature of the present study as the first attempt from India envisaged, utilization of salivary microbial diversity as an additional potential and economizing biomarker for PCOS that stimulate new horizon of research in 21st century's anthropology – the anthropology of microbes.

Keywords. diagnostic biomarker, PCOS, reproductive health, salivary microbiome, well-being

Introduction

Polycystic Ovarian Syndrome (PCOS) is one of the major reproductive health issues, currently recognized to be a multifactorial, complex endocrine disorder of less known etiology with an intricate pathophysiology.[1] It is a familial, multifaceted condition associated with different clinical manifestations (Fig. 1) such as, ovarian dysfunction, infertility, irregular menstrual cycles, anovulation, hyperandrogenism, hirsutism, acne, obesity, hypertension, diabetes mellitus, and cardiovascular abnormality, dyslipidemia, elevated pro-inflammatory cytokines, metabolic abnormalities and many conditions of the metabolic syndromes (MetS), depending on genetic background which is influenced by environmental factors.[2-10]

Furthermore, women with PCOS have a potential risk of gynecological cancer morbidities such as; endometrial cancer, ovarian cancer and breast cancer.[11,12] Thus, PCOS is considered to be not only a reproductive endocrinopathy, but also a metabolic disorder.[13] Apart from physiological maladies, studies have suggested that women with PCOS often show psychologi-

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cal issues including; symptoms of negative body image perception, low self-esteem, depression, and decreased quality of life.[14]

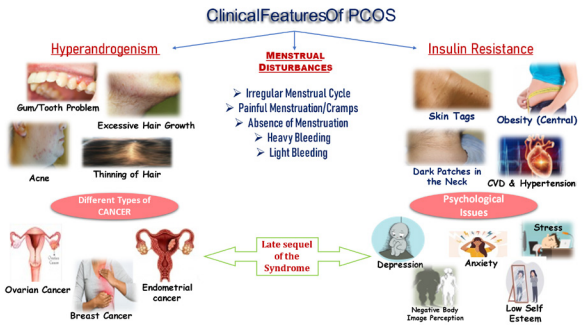

Fig. 1. Clinical manifestations of PCOS

PCOS needs serious attention because it emerges as one of the biggest health issues all over the world.[15] Globally, prevalence this syndrome is highly variable ranging from 2.2% to as high as 26%.[16,17] The prevalence of PCOS was estimated in different populations, primarily in Caucasians, black races, and a significant difference was noted in white and black females, i.e., 8% and 4%, respectively.[18-21] In Greek women, it is 6.8%. among the Chinese (East Asia) prevalence is 5.6%, while most of the studies reported among Indians (South Asia) it is nearly 9.13% to 36%. India, a developing country has witnessed a sudden rise of about 30% in PCOS cases in the last couple of years and that is only the tip of the iceberg.[22] This syndrome needs serious attention not only because it emerges as one of the biggest health issues all over the world but also as an economic burden, billions of dollars are spent annually in the United States to screen the disease.[15,23] In Indian context, it is appropriately pointed in a study that "the health budget of India is unlikely to meet the costs posed to tackle the associated multiple consequences of PCOS", needs more widespread and liberal screening for the disorder in terms of earlier diagnosis and intervention for the amelioration and prevention of early and late sequel of the disease.[23,24] Although the disease displays a wide variety of characteristics, for the diagnosis of PCOS an internationally accepted criteria is used known as, Rotterdam Criteria which requires the presence of two out of three of the following criteria: clinical/biochemical hyper androgenism, oligo-/anovulation, and polycystic ovaries.[25] Although it is a universal diagnostic criterion but it is very important to uncover some other diagnostic biomarkers along with this as this criterion is unable to discriminate the cardinal features of PCOS from various other clinical symptoms and is used when the women already developed this syndrome and suffering from its consequences but to prevent this syndrome investigation of various biomarker is much more needed to make earlier diagnosis.

Microbial population in an individual has a great impact on his overall physiological characteristics. Thus, for many clinical purpose and diagnosis, microbiota of several locations in the body has been studied and a direct correlation between a certain disease/disorder with the type, amount of microbial diversity has been established.[26] The two main components of the microecosystem are oral flora and intestinal flora.[27] Microbiome of the digestive tract has already been studied and proved to be a possible indicator of PCOS.[28] Although recent studies in abroad shows that salivary microbiome profiles are very similar with those present in gut microbiome, therefore can be used as an indicator of bacterial dysbiosis in systemic disease study.[29,30] Female Sex hormonal changes is a very important feature of PCOS and these changes are also likely to influence the salivary levels of putative periodontal pathogens (Fig. 2) causing periodontal disease like gingivitis.[31-34]

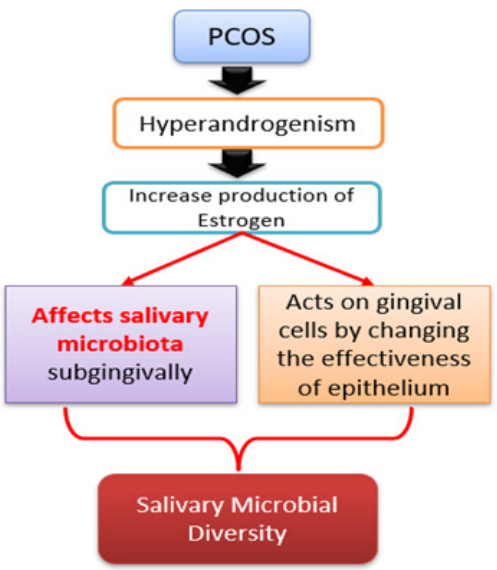

Fig. 2. Salivary microbial diversity; a biomarker of PCOS

Therefore, the levels of most of the studied putative periodontal pathogens can be studied as a biomarker of PCOS. Although there are still limited studies in this regard, but a contemporary study from abroad reported PCOS could quantitatively affect the composition of the salivary microbiota and the relative abundance of salivary Actinobacteria was reduced in PCOS patients compared with healthy controls.[27,30,31] However, no such report has been found from India yet. The anthropology of microbes can expand ethnographic analyses to include investigations of "indigenous" microbial populations (microbiota) and shaping human health and their possible impacts in clinical practice.[35] Thus, apart from the conventional Rotterderm Criteria salivary microbial diversity study can also be taken as a useful diagnostic biomarker for PCOS studies.

Aim

In this backdrop, the present study, to best of the knowledge is the first attempt, to discern the association of salivary microbial diversity between PCOS and non PCOS individuals.

Material and methods

Present study was conducted among one hundred clinically diagnosed PCOS patients, selected from 'Ramkrishna Mission Seva Pratisthan (Shishumangal)', Kolkata, under the assistance of Gynecology department and one hundred and ten (110) non PCOS individuals from the Department of Microbiology, Department of Biochemistry and Department of Anthropology, University of Calcutta, Ballygunge campus. Prior to the study verbal and written consent were obtained from the participants.

The saliva samples were collected from all the 210 persons (PCOS and non PCOS) using commercially available Sterile Cotton Swab Stick. Then the samples were inoculated into L-B broth (2%). Total DNA was extracted from saliva samples using a standardized protocol[30] with slight modification. A PCR reaction was performed to amplify the V3–V4 region of the bacterial 16S rRNA gene using the primers 27 F primer (AGAGTTTGATCMTGGCTCAG) and 1492 R primer (GGTTACCTTGTTACGACTT) with initial denaturation at 95^{0}C for 5 minutes, followed by 35 cycles of denaturation at 95 ^{0}C for 30 seconds, annealing at 50^{0}C for 30 seconds, and extension at 72^{0}C for 1.5 minutes, one cycle of final extension at 72^{0}C for 10 minutes and final cooling step to 4^{0}C. The PCR samples were run on 1% agarose gel to visualize the bands of 16S rRNA (V3–V4) region under UV transilluminator. For genotyping, RFLP were done using a tetra cutter restriction endonuclease - Alu I which cuts at the sequence 5' AG$^{\vee}$CT 3'. Then the amplified 16S DNA were subjected to restriction digestion and gel electrophoresis was performed using 2% agarose gel, visualized under UV Trans illuminator.

Results

The graphical representation using the fragment lengths of PCOS and non PCOS after treating them with Alu I shows that the respective restriction enzyme cut the fragments of length 200-225 bp (Fig. 3) for those with PCOS while, women with no signs of polycystic ovary showed no particular pattern of DNA fragment lengths. Their fragment lengths varied from 100 bp to 550 bp (Fig. 4). Almost 70% showed a similar fragment length (100 bp and 200 bp) indicating significant pattern (Fig. 5) which establishes the fact that there might be a prevalence of a particular group of bacteria in the oral cavity of women having PCOS.

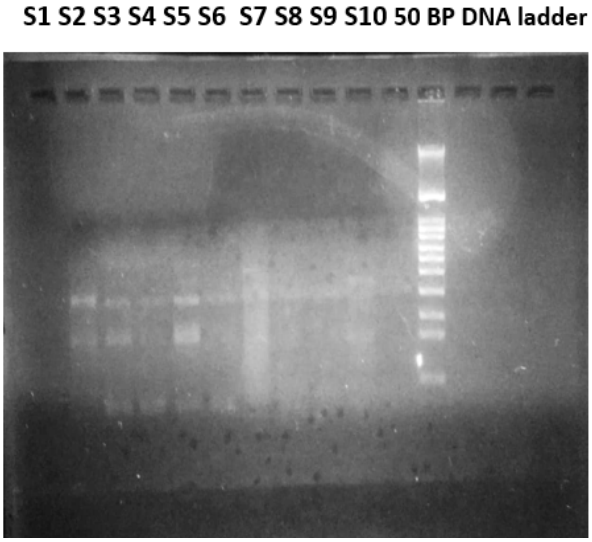

Fig. 3. DNA fragments of PCOS samples after being digested with Alu I

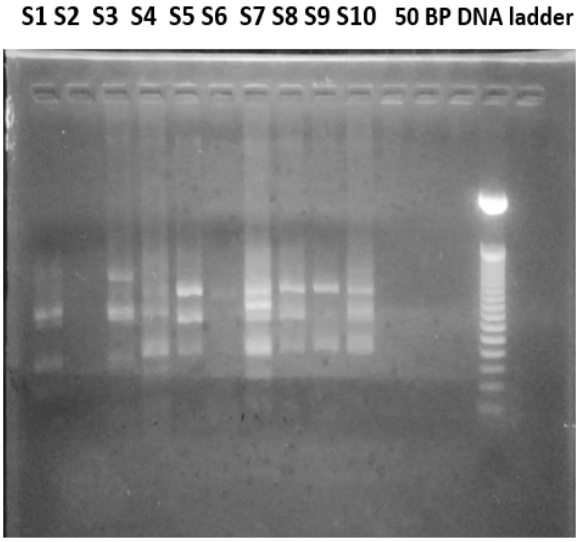

Fig. 4. DNA fragments of NON-PCOS samples after being digested with Alu I

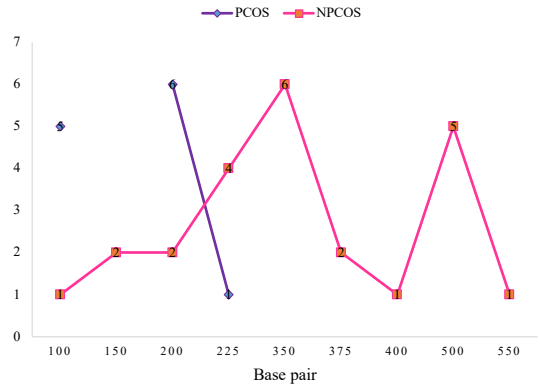

Fig. 5. Relationship between different fragment lengths of PCOS and Non-PCOS after treatment with Alu I

Discussion

It is apparent from the foregoing study, PCOS is an endocrine disorder affecting reproductive-aged women, but the cause remains unclear. However, in a recent systematic review and meta-analysis on current knowledge of the microbes across body sites (oral cavity, blood, vagina/cervix, gut)" in PCOS, it was reported that there is growing evidence links oral and salivary microbial composition to PCOS.[30,31,27,37] Most studies (around 75%) discerned low-risk bias, but incidentally, all the studies were conducted from abroad and eventually, the present study is perhaps the single and first reported evidence from India. Female sex hormone levels have been associated with oral microbiome composition linked to oral pathologies, such as periodontal disease, which indicates the plausible effect of oral microbial organization.[36] However, interestingly, lower alpha oral microbial diversity in PCOS was noticed compared with controls and the present study also in consistency when, Alu I showed that the respective restriction enzyme cut the fragments of length 200-225 bp among the PCOS, in comparison to much diverse as from 100 bp to 550 bp for non-PCOS.[27] Since entire participants of the present study were from a single ethnic population, therefore, minimize the confounder effects of endogenous and exogenous factors, such as oral hygiene, diet, smoking (none), drinking habit (none) was found to have in similarity in the PCOS and without PCOS participants. Most human studies have determined differences in microbial taxa in women with and without PCOS.[38,39] As mentioned earlier the systematic review and meta-analysis reported a significant pooled Standardized Mean Difference (SMD) value (detected for Shannon diversity index), indicating a significantly lower bacterial richness in PCOS individuals compared with the non-PCOS control group and the present study, best of the knowledge, the first report from India found in corroboration of lower oral microbiome diversity in PCOS compared to the non-PCOS group counterpart.[37] Therefore, the salivary microbial diversity study can be taken as a useful diagnostic biomarker of reproductive health studies.

Conclusion

This is the first report of the salivary microbiome diversity in PCOS sample from India. This data may become helpful for clinical studies and diagnosis of PCOS prior to USG. As, analyzing salivary microbiome is more convenient than gut microbiota. This study opens a new door to the medical field to carry forward the research on polycystic ovary syndrome which is largely affecting almost every other woman in this world. However, several other studies should be conducted with other digestive enzymes and large sample size to ensure this result and to further clarify the relationship between salivary microbiota and PCOS.

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Declarations

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Author contributions

Conceptualization, K.G., S.C., D.C. and A.R.B.; Methodology, K.G. and S.C.; Software, K.G. and S.C.; Validation, K.G., S.C. and A.R.B.; Formal Analysis, K.G., S.C. and A.R.B.; Investigation, K.G., S.C. and A.R.B.; Resources, K.G., S.C. and A.R.B.; Data Curation, K.G. and S.C.; Writing – Original Draft Preparation, K.G., S.C. and A.R.B.; Writing – Review & Editing, K.G., S.C., D.C., and A.R.B.; Visualization, K.G. and S.C.; Supervision, A.R.B.; Project Administration, A.R.B. and D.C.; Funding Acquisition, A.R.B..

Conflicts of interest

No conflict of interest.

Data availability

Data available on request from the authors.

Ethics approval

Institutional Ethical Committee for Bio Medical and Health Research involving Human Participants, University of Calcutta & Secretary, UCSTA, C.U., Ref no.: CUIEC/02/15/2022-23.

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