Original Article

Unveiling Therapeutic Potential of Yoga Mitigating Oxidative Stress and Mitochondrial Dysfunction in PCOS: A Randomized Controlled Trial

Deepika Kumari, Manoj Kumar, Ashish Datt Upadhyay[1], Neena Malhotra[2], Reeta Mahey[2], Vatsala Dadhwal[2], Tushar Sehgal[3], Richa Mishra, Rima Dada

Department of Anatomy, Laboratory for Molecular Reproduction and Genetics, AIIMS, Departments of [1]Clinical Research Unit (CRU), [2]Laboratory Medicine and [3]Obstetrics and Gynaecology, AIIMS, New Delhi, India

Abstract

Background: Hormonal imbalance, mitochondrial dysfunctions, and oxidative stress (OS) have been implicated in the pathogenesis of polycystic ovarian syndrome (PCOS) and its associated clinical features. A sedentary lifestyle, exposure to air pollutants, prenatal exposure to endocrine-disrupting chemicals, processed and nutritionally depleted food, rich in trans fats, salts, and sugars, and high BMI specifically in visceral adiposity induce OS. OS damages the mitochondrial DNA, lipids, and proteins that impair mitochondrial function. Sequentially, dysfunctional mitochondria produce more reactive oxygen species that aggravate the OS. Mitochondria is pivotal for ovarian cell functioning for instance steroidogenesis, ovarian follicle development, and energy metabolism. Dysfunctional mitochondria can alter the ovarian follicle functioning leading to ovulatory dysfunction and infertility in PCOS. **Aims and Objectives:** This study is designed to investigate the effect of 12-week yoga practice on endocrine parameters, OS, and mitochondrial health, comparing outcomes in yoga and non-yoga groups. **Material and Methods:** A total of 75 participants, 32 PCOS females who completed yoga intervention in the yoga group and 29 in the nonyoga group. Hormonal levels were assessed through an immunoassay, while mitochondrial health markers, such as mtDNA copy number (mtDNA CN), reactive oxygen species, and lipid peroxidation were measured through quantitative polymerase chain reaction (qPCR), chemiluminescence, and ELISA respectively. Gene expression related to mitochondrial integrity, respiratory chain, and inflammation was analyzed via reverse transcription qPCR. Additionally, depression severity was also assessed using beck depression inventory II. **Result:** The Yoga group showed a significant increase in mtDNA-CN and upregulation of transcripts responsible for maintaining mitochondrial integrity and the mitochondrial respiratory chain. In addition, the post-yoga group shows a reduction in, lipid peroxidation, inflammatory, OS markers, and an improvement in telomere length. **Conclusion:** Yoga positively affects hormonal balance, mitochondrial health, OS, and inflammation in women with PCOS. It also alleviates depression symptoms, highlighting yoga as an effective adjunct therapy for managing PCOS. Regular yoga practice could prevent, delay, and help in managing PCOS symptoms.

Keywords: *Inflammation, micronanoplastics, mitochondrial dysfunction, oxidative stress, PCOS, plastics, yoga*

Introduction

Polycystic ovarian syndrome (PCOS) is one of the most common endocrinopathies among reproductive age females, with a 4%–20% prevalence.[1] It is increasing in the urban and rural areas due to unhealthy and sedentary lifestyles, including consuming processed foods, rich with salt, sugar, trans fats, and no or less physical activity. The most acceptable diagnostic tool for PCOS is the Rotterdam criteria. It needs two of the following clinical features for confirmation: oligoovulation/anovulation (O), hyperandrogenism (HA), and polycystic ovarian morphology (PCOM).[2] Its specific etiology is unknown. Studies suggest that an interaction of genetic, epigenetic, and environmental factors causes PCOS.[3] In addition, exposure to micronanoplastics (MNP) either through consumption of food, water, or inhalation from air can adversely affect oogenesis. MNPs like bisphenol A and phthalates and heavy metals have various endocrine-disrupting chemicals that are known to decrease ovarian reserve damage granulosa cells (GCs) and disrupt the hypothalamo pitutary gonadal axis.[4,5] A sedentary lifestyle, unhealthy social behaviors, eating fast food and ultra-processed foods rich in sugars and carbohydrates, and resultant high basal mass index (BMI) also lead to PCOS. It is a lifestyle disorder that is associated with metabolic

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Address for correspondence:
Prof. Rima Dada, Room No. 1036, Department of Anatomy, All India Institute of Medical Sciences, New Delhi - 110 029, India. E-mail: rimadadaaiims20@gmail.com

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syndrome, nonalcoholic fatty liver disease (NAFLD), ovarian and endometrial cancers, cardiovascular disease (CVD), and Alzheimer's. Hence, its early diagnosis is crucial for the management and preventing the sequelae and a better prognosis.

HA is a crucial feature that is exhibited in 65%–80% of PCOS women.[6] In PCOS, gonadotropin-releasing hormone pulsatility is high, which leads to excess luteinizing hormone (LH) secretion. Elevated LH and lower follicle-stimulating hormone (FSH) levels cause HA, due to the hypersensitivity of theca cells to LH and incomplete androgen aromatization in GCs of the ovarian follicle.[7] In addition, a high level of androgens recruits more primordial follicles that release Anti-Müllerian hormone (AMH). In PCOS, circulating AMH levels are elevated two to three-fold and positively correlated with PCOM and HA. Furthermore, It inhibits dominant follicle selection and maturation leading to ovulatory dysfunction.[8] High androgens level causes hirsutism, acne, androgenic alopecia, and weight gain. These body image appearances can exacerbate depression onset in individuals with PCOS.[9]

Despite the exact trigger, PCOS women have high-reactive oxygen species (ROS) and reduced antioxidant levels regardless of clinical features such as obesity, dyslipidemia, HA, and hyperinsulinemia.[10-12] ROS are known for their dual nature in both constructive and destructive effects. At the physiological level, ROS are crucial in regulating redox reactions to maintain cellular equilibrium.[13] At the supraphysiological level, ROS causes oxidative stress (OS), a harmful process that damages mitochondrial and nuclear DNA, lipids, and proteins. PCOS women have significantly lower mitochondrial DNA copy number (mtDNA-CN) than healthy controls.[14] Numerous chronic lifestyle disorders such as certain cancers, CVD, and autoimmune disorders are linked with lower mtDNA-CN.[15,16] The mitochondrial and nuclear genomes are important in generating and regulating cellular energy.[17] This damage at the cellular and mitochondrial level also contributes to metabolic and hormonal imbalances seen in PCOS.[18] Dysfunctional mitochondria lead to inefficient electron transport chain and oxidative phosphorylation (OXPHOS), defective mitophagy, and increased fatty acid reflux in turn, increases ROS production.[19] Cells have defense systems to combat excessive ROS using antioxidants, while imbalance leads to OS. In addition, OS can cause peroxidative damage that changes the activity of mitochondrial integrity, and mitochondrial respiratory chain (MRC) functioning.[20,21] It is one of the etiological factors in various reproductive disorders such as PCOS, endometriosis, unexplained infertility, and preeclampsia.[22-24]

Besides, OS affects the nuclear genome and leads to telomere shortening that is associated with PCOS. Telomeres are repeating TTAGGG sequences at the ends of chromosomes, gradually getting shorter as cells divide. Previous studies have demonstrated the significance of telomere length shortening in several illnesses, including PCOS, diabetes, cancer, and CVD.[25-28] Correspondingly, ROS molecules have the potential to dysregulate certain signaling pathways including c-Jun N-terminal kinases (JNK), Nuclear Factor kappa B (NF-κB), Phosphatidylinositol 3-kinase/protein kinase B (Akt), and mitogen-activated protein kinases that is responsible for inflammation in PCOS.[29] Low-grade chronic inflammatory cytokines, like Interleukin-6 (*IL-6*) and tumor necrosis factor-alpha (*TNF-α)* pivotal contributors to PCOS.[30] *IL-6* is an important cytokine in inflammation and altered immune responses. Its elevated level is linked with metabolic and reproductive alteration in PCOS.[31] *TNF*-α mostly released by the macrophages intervenes in the insulin signaling pathway that eventually leads to insulin receptor (IR).[32] In addition, OS can damage germ cells resulting in poor oocyte quality, reduced fertilization ability, and chromosomal missegregation during meiosis, which can lead to ovulatory dysfunction, infertility, and HA.[33] Furthermore, free radicals' oxidative destruction of lipids leads to lipid peroxidation, which can damage the cell membranes. Therefore, to maintain cellular health careful homeostasis of OS is essential.

OS can alter the genetics and epigenetics of a cell. In a stressful lifestyle, optimizing OS levels is challenging, and careful control is needed. On the contrary, the effects of OS on cells may be reduced by therapies like antioxidant therapy and lifestyle changes. Furthermore, lifestyle modifications are considered first-line management for PCOS. Although synthetic antioxidants can combat OS, their indiscriminate use may lead to "reductive stress." In addition, high-intensity physical exercise elevates free radicals release, lipid peroxidation, and DNA damage, along with stimulation of the sympathetic nervous system.[34-36] Previous studies revealed yoga alleviates OS, improves DNA damage repair, and stimulates parasympathetic nervous system activity.[37,38] Yoga has positive effects on numerous chronic conditions including major depressive disorder, rheumatoid arthritis, infertility, and CVDs.[39-44] To the best of our knowledge, the effects of yoga on cellular health in PCOS, are not well known. It is crucial to study the impact of yoga and its potential as an adjunct therapy for PCOS treatment. Hence, this study aimed to investigate the effect of a 12-week yoga intervention on individuals diagnosed with PCOS. The primary objectives of this study were to evaluate OS (ROS, 8-hydroxy-2'-deoxyguanosine [8oHdG], and total antioxidative capacity [TAC]), inflammatory parameters (*TNF-α,* and IL-6), aging parameters (cyclooxygenase-2 [COX-II], NAD+, and telomere length), mtDNA-CN, mitochondrial integrity (*adenosine monophosphate-activated protein kinase [AMPK], SIRT1, transcription factor A mitochondrial [TFAM],* and *nuclear respiratory factor 1 [NRF1]*) and transcripts associated with MRC (*NADH: Ubiquinone Oxidoreductase Subunit A3 [NDUFA3], Succinate Dehydrogenase Subunit D [SDHD], Cytochrome c Oxidase Subunit 7C [COX7C],* and *ATP5PD*) in both yoga and nonyoga group. The secondary objective was to evaluate depression severity in both groups.

Materials and Methods

Participants

The PCOS females were referred from the obstetrics and gynecology department's outpatient unit at our institute. Randomization was done using the research randomizer web tool (https://www.randomizer.org/). The inclusion criteria included the age group of 18–40 years' PCOS patients who met the criteria outlined in the 2003 ESHRE/ASRM (Rotterdam criteria).[45] PCOS women who have other causes of hyperandrogenemia and anovulatory dysfunction and are physically challenged to perform the postures were excluded.

Study design

It was a randomized controlled trial to assess the 12-week yoga effect on OS, inflammation, aging, and mitochondrial integrity in PCOS women. This study was started after obtaining institute ethical clearance (IEC-606/15.07.2022) and clinical trial registration (CTRI/2022/10/046761) from the institutional ethics committee and the clinical trial registry of India, respectively. Before starting of intervention, each patient has signed informed consent.

Sample size

Based on previous research that shows the positive effect of yoga on PCOS women, similar sample size we have considered for this study.[46] Then, the sample size was assessed based on the following criteria; alpha at 0.05, powering at 0.8 for a medium effect size, and the attrition rate at 15%–20%, following past yoga studies.[39] Furthermore, we have considered some loss to follow-up and decided to recruit 38 PCOS patients for the yoga group and 37 for the nonyoga group (total n = 75).

Intervention

Before the intervention, baseline characteristics and history were taken. Nonyoga groups have been advised to be active and exercise (brisk walking) at home at least 5 days a week for an hour for 12 weeks. Yoga group participants underwent 5 days a week for a 12-week yoga intervention in the anatomy department, All India Institute of Medical Sciences, New Delhi, by a yoga therapist. The practice sessions were incorporated from the Patanjali yoga sutra including Asanas (physical postures), Pranayama (regulated breathing practices), and Dhyan (Meditation) for approximately 1 ½ h [Table 1].

Primary outcome

Mitochondrial DNA copy number analysis

The genomic DNA was used for mtDNA copy number. Genomic DNA is isolated from whole blood through the salting out manual method.[47] Furthermore, the relative ratio of the mitochondrial NADH -ubiquinone oxidoreductase chain 1 (ND1) and 36B4 gene was determined using qPCR. This ratio is directly related to the average number of mtDNA-CN. The primer sequences are given in Table 2.

Telomere length analysis

Telomere length was measured through qPCR using genomic DNA. The expression levels of the single-copy gene (S) and telomere repeat sequence (T) were measured to determine the relative telomere length. The T/S ratio was then calculated for each patient individually.

Table 1: Details of a yoga therapy session on a single day

Practice to be done	Duration (min)
Prayer	2
Yogic sukhsmavyayam	
Loosening fingers and wrists, easing tension in elbows, relieving shoulders, flexing toes, mobilizing ankles, bending knees, and rotating hips	3
Yogic sthulavyayam	
Sarvang Pushti	6
Hridgati	
Suryanamaskar	
Yoga Asana	
Standing	
Tadasana	5
Tiryaktadasanaa	
Katichakrasana	
Sitting	
Malasana	10
Baddha Konasana	
Yoga Mudrasana	
Chakkichalan asana	
Janusirs asana	
Ushtra asana	
Brahmachary asana	
Marjariasana	
Prone	
Bhujangasana	5
Dhanurasana	
Nauka asana	
Supine	
Supta badhakan asana	5
Uttanapadasana	
Markatasanaand	
Shavasana	
Pranayam	
Kapalbhati	25
Bhastrika	
Nadishoadhna	
Bhramri	
Meditation	
Breathing with awareness of breath	5
Prayer with Shanti mantra	4
Interactive discussion	20
Total	90

Table 2: List of primers and their sequence

Genes	Primer sequence (5'–3')
AMPK	Forward: TTTGCGTGTACGAAGGAAGAAT
	Reverse: CTCTGTGGAGTAGCAGTCCCT
SIRT1	Forward: AGCCTTGTCAGATAAGGAAGGA
	Reverse: ACAGCTTCACAGTCAACTTTGT
TFAM	Forward: GTTGGAGGGAACTTCCTGATT
	Reverse: CTGACTTGGAGTTAGCTGTTCT
NRF1	Forward: TGATGGCACTGTCTCACTTATC
	Reverse: ATCAGCCACGGCAGAATAAT
NDUFA3	Forward: CTCAAGAATGCCTGGGACAA
	Reverse: CCTTGTTGATCATGACGGAGTA
SDHD	Forward: GCTGCATCTCTCCACTG
	Reverse: CCAGTGACCATGAAGAGTGAG
COX7C	Forward: GTCCGTAGGAGCCACTATGA
	Reverse: GTGTAGCAAATGCAGAT
ATP5PD	Forward: ATTGCTAGTTCCCTGAAATCCT
	Reverse: TTGTAGTAAGCCCAGTCGATAG
IL-6	Forward: GGCACTGGCAGAAAACAACC
	Reverse: GCAAGTCTCCTCATTGAATCC
TNF alpha	Forward: ACCTCCGAGATGACACCATCA
	Reverse: GGCACTCTGGCACATATTCAC
MT-ND1	Forward: ACCTCCTACTCCTCATTGTAC
	Reverse: GTTCATAGTAGAAGAGCGATG
Telomere length	Forward: GTTTTTGAGGGTGAGGGTGAGGGT GAGGGTGAGGGT
	Reverse: TCCCGACTATCCCTATCCCTATCCCTA TCCCTATCCCTA
36B4	Forward: AACATGCTCAACATCTCCCC
	Reverse: CCGACTCCTCCGACTCTTC

AMPK: Adenosine monophosphate-activated protein kinase, TFAM: Transcription factor a mitochondrial, NRF1: Nuclear respiratory factor 1, NDUFA3: NADH: Ubiquinone Oxidoreductase Subunit A, SDHD: Succinate dehydrogenase Subunit D, COX7C: Cytochrome c Oxidase Subunit 7C, IL-6: Interleukin-6, TNF-α: Tumor necrosis factor-alpha

Real-time-quantitative polymerase chain reaction for gene expression

Peripheral blood mononuclear cells (PBMCs) were isolated using ficoll gradient method. From PBMCs, total RNA was isolated using TRIzol. One microgram RNA reverse transcribed to produce complementary DNA (cDNA). Each gene expression was quantitatively analyzed (CFX96 real-time equipment, Bio-Rad, CA, USA) and the Brilliant III Ultra-Fast SYBR Green qPCR Master Mix. For the normalization internal housekeeping genes were used and cDNA products were examined in triplicate. In addition, each gene was amplified for 35 cycles. The $2^{-\Delta\Delta Ct}$ technique was used for the relative quantification of target transcript expression. All the primer sequences are included in Table 2.

Enzyme-linked immunosorbent assay

An enzyme-linked immunosorbent assay kits for the following were used 8OHdG (Cayman's EIA ki), 4HNE (KRISHGEN biosystems Cerritos, USA), TAC (Cayman Chemical, Ann Arbor, USA), and NAD+ (My Biosource, Inc., USA). Assays for quality control and validation of biochemical indicators were carried out. Absorbance was measured at 450 nm.

Assay for cyclooxygenase-2 activity

Mitochondria were isolated from PBMC through a mitochondria isolation kit (BioVision, CA, USA). Furthermore, a colorimetric test kit for COX-II activity (cytochrome c oxidase, BioVision, CA, USA) was used with absorbance measured at 550 nm.

Chemiluminescence assay

For the ROS analysis, we have used 10 µL of a 5 mM luminol solution (5-amino-2,3-dihydro-1,4-phthalazinedione, Sigma Chemical Co., St. Louis, MO, USA) for 400 µL of heparinized whole blood. Then, we recorded the chemiluminescence for 10 min. When ROS released by the neutrophils in the entire blood interact with luminol, which acts as a chemiluminescent probe, an excited reaction intermediate is produced. When this intermediate product reaches its ground state again, it releases light. Negative and positive control was prepared by mixing 10 µL of a 5 mM luminol solution with 400 µL of phosphate-buffered saline hydrogen peroxide, respectively, to provide baseline value. Each run was carried out twice, and the relative light units per minute per 10^4 neutrophil counts (RLU/10^4/N), which takes into account neutrophils as the main producers of ROS production in the blood, was used to quantify the average values that were obtained.

Immunoassay

To analyze the following hormone levels; FSH, LH, AMH, and total testosterone immunoassay were used. Serum samples were used for the hormonal analysis and were separated from blood by centrifugation. It was stored at a temperature of − 20°C after collection till further processing.

Flow cytometry analysis for mitochondrial membrane potential

Fresh PBMCs were analyzed using JC-10 dye. The cells were incubated with the dye for 30 min at 37°C before proceeding to flow cytometry. JC-10, a cationic dye, permeates healthy mitochondria and forms reversible aggregates that emit red fluorescence. In apoptotic cells, JC-10 is present in its monomeric form, emitting green fluorescence. A decrease in the red-to-green fluorescence intensity ratio indicates mitochondrial membrane depolarization, while an increase suggests membrane polarization.

Secondary outcome

Depression severity

The severity of the depression score was evaluated through the Beck Depression Inventory-II tool. It is a robust tool that includes 21 multiple-choice questions; each question

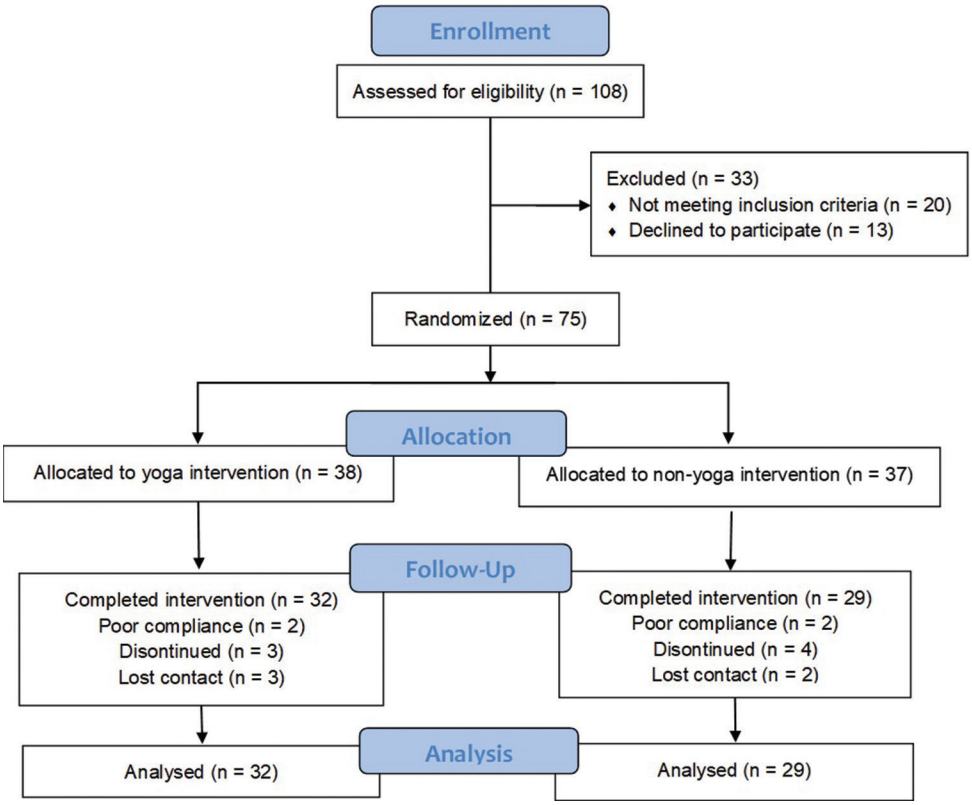

Figure 1: A CONSORT flow diagram

has four responses. Each question score ranges between 0 and 3 (total 0–63).

Data analysis

For all the statistical data analyses, Graph Pad Prism (Version 8.0.1. GraphPad Software Inc., California, USA) was used. Descriptive statistics are described as mean ± standard deviations. The paired-sample *t*-test was applied for the outcome variables within the group while the unpaired sample *t*-test was used for between-group analysis. Significance was considered at a $P < 0.05$.

Results

Figure 1 depicts the consort flow of participation. A total of 108 individuals underwent eligibility screening, of which 75 were randomly assigned to receive the intervention. Among these, 38 were allocated to the yoga group and 37 to the nonyoga group. Within the yoga group, 6 participants left while in the nonyoga group, 11 participants dropped out due to poor compliance, with some discontinued and lost contact. Ultimately, 32 patients from the yoga group and 29 in the nonyoga group completed the intervention and analyzed for the outcomes.

Primary outcome

Baseline sociodemographic characteristics

The baseline sociodemographic characteristics are shown in Table 3.

Table 3: Baseline sociodemographic characteristics and data represented as mean±standard deviation

Variable	Yoga group (mean±SD)	Nonyoga group (mean±SD)
Age (years)	23±4.410	23.900±3.630
Height (m)	1.560±0.068	1.540±0.068
Weight (kg)	62.381±13.108	62.897±11.195
BMI (kg/m^2)	25.728±5.586	25.578±4.779
Kuppuswamy socioeconomic status scale		
Occupation	6.160±2.400	6.690±2.050
Education	4.720±1.870	4.860±1.600
Income	6.630±3.640	6.900±3.020
Total	17.500±6.740	18.480±4.880

BMI: Body mass index, SD: Standard deviation

Postyoga differences in laboratory indicators

Anthropometric and hormonal parameter

A significant reduction in BMI (yoga group [0.685, 95% confidence interval (CI) (0.265–1.104)]), (nonyoga group [−1.159, 95% CI (−2.098 to − 0.220)]) was found within both yoga and nonyoga groups, while nonsignificant at baseline and post 12 weeks (between groups). There was a significant reduction in the hormonal parameters such as LH (1.251, 95% CI [0.328–2.174]), LH/FSH ratio (0.962, 95% CI [0.473–1.450]), and AMH (1.721, 95% CI [0.559–2.882]) within the yoga group and post

12 weeks (between groups), while testosterone (1.436, 95% CI [0.234–2.638]) was significantly reduced only in the yoga group. In addition, significant elevation was seen in FSH (−0.775, 95% CI [−1.296 to − 0.254]) level after 12 weeks of yoga practice and nonsignificant baseline and post-12 weeks (between groups) [Table 4].

Oxidative stress parameters

As depicted in Table 4, there was a significant reduction in the ROS (312.000, 95% CI [125.600–498.400]), 4HNE (102.500, 95% CI [47.820–157.300]), and 8OHdG (112.200, 95% CI [30.810–193.700]) levels, whereas the

Table 4: Results of the within-group and between the analysis of study outcomes, data represented as mean±standard deviation

Outcome measure	Characteristics	Yoga group	Nonyoga group	*P* between groups
BMI (kg/m^2)	Baseline measurement (mean±SD)	25.728±5.586	25.578±4.779	0.911
	Post 12-week measurement (mean±SD)	25.043±4.973	26.737±3.942	0.149
	Change from baseline to 12 weeks, mean (95% CI)	0.685 (0.265–1.104)	−1.159 (−2.098–−0.220)	
	P value within group	0.002	0.017	
FSH (mIU/mL)	Baseline measurement (mean±SD)	4.030±1.355	4.052±1.253	0.948
	Post 12-week measurement (mean±SD)	4.805±1.112	4.312±1.267	0.111
	Change from baseline to 12 weeks, mean (95% CI)	−0.775 (−1.296–−0.254)	−0.260 (−0.819–0.299)	
	P value within group	0.004	0.349	
LH (mIU/mL)	Baseline measurement (mean±SD)	12.643±1.851	12.312±1.277	0.426
	Post 12-week measurement (mean±SD)	11.392±1.445	12.327±2.148	0.049
	Change from baseline to 12 weeks, mean (95% CI)	1.251 (0.328–2.174)	−0.014 (−1.050–1.022)	
	P value within group	0.010	0.978	
LH/FSH ratio	Baseline measurement (mean±SD)	3.468±1.246	3.335±1.138	0.665
	Post 12-week measurement (mean±SD)	2.507±0.691	3.103±1.205	0.020
	Change from baseline to 12 weeks, mean (95% CI)	0.962 (0.473–1.450)	0.232 (−0.377–0.841)	
	P value within group	<0.001	0.442	
AMH (ng/mL)	Baseline measurement (mean±SD)	12.113±3.325	11.980±2.735	0.865
	Post 12-week measurement (mean±SD)	10.393±1.764	11.733±1.691	0.004
	Change from baseline to 12 weeks, mean (95% CI)	1.721 (0.559–2.882)	0.246 (−0.855–1.347)	
	P value within group	0.005	0.650	
Testosterone (ng/dL)	Baseline measurement (mean±SD)	36.551±6.465	37.288±5.670	0.640
	Post 12-week measurement (mean±SD)	35.116±5.914	36.606±6.151	0.340
	Change from baseline to 12 weeks, mean (95% CI)	1.436 (0.234–2.638)	0.682 (−1.703–3.067)	
	P value within group	0.021	0.599	
ROS (RLU/min/104 neutrophils)	Baseline measurement (mean±SD)	957.592±765.116	1026.466±811.293	0.73
	Post 12-week measurement (mean±SD)	645.633±532.921	1325.516±525.637	<0.001
	Change from Baseline to 12 weeks, mean (95% CI)	312 (125.600–498.400)	−299.100 (−621.800–23.710)	
	P value within group	0.001	0.068	
8OHdG (pg/mL)	Baseline measurement (mean±SD)	1024.419±89.033	1090.699±260.337	0.180
	Post 12-week measurement (mean±SD)	912.180±202.465	1162.089±300.941	<0.001
	Change from baseline to 12 weeks, mean (95% CI)	112.200 (30.810–193.700)	−71.390 (−205–62.210)	
	P value within group	0.009	0.283	
4 HNE (pg/mL)	Baseline measurement (mean±SD)	273.783±109	272.968±108.681	0.977
	Post 12-week measurement (mean±SD)	171.243±72.152	219.640±108.834	0.043
	Change from baseline to 12 weeks, mean (95% CI)	102.500 (47.820–157.300)	53.330 (−17.030–123.700)	
	P value within group	<0.001	0.131	

Contd...

Table 4: *Contd...*

Outcome measure	Characteristics	Yoga group	Nonyoga group	*P* between groups
TAC (mmol Trolox equiv/L)	Baseline measurement (mean±SD)	5.605±0.725	5.332±0.779	0.160
	Post 12-week measurement (mean±SD)	6.030±0.988	5.309±0.780	<0.001
	Change from baseline to 12 weeks, mean (95% CI)	−0.425 (−0.740–−0.110)	0.023 (0.200–0.241)	
	P value within group	0.010	0.832	
NAD + (ng/mL)	Baseline measurement (mean±SD)	1478.402±381.344	1426.459±119.384	0.49
	Post 12-week measurement (mean±SD)	1651.130±107.490	1496.329±199.246	0.0003
	Change from baseline to 12 weeks, mean (95% CI)	−172.700 (−308.200–−37.270)	−69.870 (−144–4.259)	
	P value within group	0.01	0.06	
COX-II (unit/mg mito protein)	Baseline measurement (mean±SD)	1.940±0.611	1.840±0.411	0.460
	Post 12-week measurement (mean±SD)	2.500±0.590	1.918±0.490	<0.001
	Change from baseline to 12 weeks, mean (95% CI)	−0.560 (−0.861–−0.258)	−0.078 (−0.250–0.093)	
	P value within group	<0.001	0.360	
MMP (red/green fluroscence)	Baseline measurement (mean±SD)	3.826±1.547	3.764±1.562	0.560
	Post 12-week measurement (mean±SD)	5.020±2.020	4.188±1.790	0.044
	Change from baseline to 12 weeks, mean (95% CI)	−1.193 (−2.013–−0.3739)	−0.171 (1.033–0.691)	
	P value within the group	0.006	0.689	
Mt-DNA copy number	Baseline measurement (mean±SD)	1.195±0.105	1.188±0.122	0.980
	Post 12 week measurement (mean±SD)	1.299±0.051	1.209±0.122	<0.001
	Change from baseline to 12 weeks, mean (95% CI)	−0.104 (−0.143–−0.065)	−0.02 (−0.063–0.018)	
	P value within group	<0.001	0.268	
Telomere length	Baseline measurement (mean±SD)	0.799±0.052	0.799±0.058	0.984
	Post 12 week measurement (mean±SD)	1.035±0.103	0.831±0.082	<0.001
	Change from baseline to 12 weeks, mean (95% CI)	−0.236 (−0.271–−0.201)	0.032 (−0.006–0.700)	
	P value within group	<0.001	0.093	
BDI	Baseline measurement (mean±SD)	17.594±6.705	17.380±6.321	0.899
	Post 12 week measurement (mean±SD)	11.594±6.211	15.966±4.633	0.003
	Change from baseline to 12 weeks, mean (95% CI)	6 (3.411–8.589)	1.414 (−0.448–3.276)	
	P value within group	<0.001	0.131	

BMI: Body mass index, SD: Standard deviation, CI: Confidence interval, BDI: Beck depression inventory, MMP: Mitochondrial membrane potential, COX-II: Cyclooxygenase-2, NAD: Nicotinamide adenine dinucleotide, TAC: Total antioxidative capacity, 4HNE: 4-hydroxynonenal, LH: Luteinizing hormone, FSH: Follicle-stimulating hormone, AMH: Anti-Müllerian hormone, RLU: Relative light units, 8OHdG: 8-hydroxy-2'-deoxyguanosine, ROS: Reactive oxygen species

TAC (−0.425, 95% CI [−0.740 to − 0.110]) level showed a significant elevation within the yoga group and after 12 weeks (between groups) [Table 4].

Mitochondrial integrity and mitochondrial respiratory chain indicators

MtDNA-CN exhibits a significantly higher CN within the yoga group (−0.104, 95% CI [−0.143 to − 0.065]) and post 12 weeks (between groups) [Figure 2]. Furthermore, mitochondrial membrane potential (MMP) showed a significant increase in the yoga group (−1.193, 95% [−2.013 to − 0.3739]) and post 12 weeks in between groups [Table 4]. The mean axis fold change of mitochondrial integrity and MRC transcripts was as follows in the yoga as compared to nonyoga group: *AMPK* (2.060 vs. 1.910), sirtuin-1 (SIRT-1) (2.730 vs. 0.370), *TFAM* (5.190 vs. 0.280), *NRF1 (*4.550 vs. 1.620), *NDUFA3 (*5.570 vs. 1.880), SDHD (5.770 vs. −0.040), *COX7C* (3.440 vs. 0.330), and *ATP5PD* (4.450 vs. 0.460) [Figure 3].

Inflammatory transcripts

The expression of the inflammatory transcripts TNF-α (−2.230 vs. −1.440) and IL-6 (−2.140 vs. 0.490) was downregulated in the yoga versus nonyoga group [Figure 3].

Aging marker

The telomere length exhibited a significant elevation within the yoga group (−0.236, 95% CI [−0.271 to − 0.201]) and

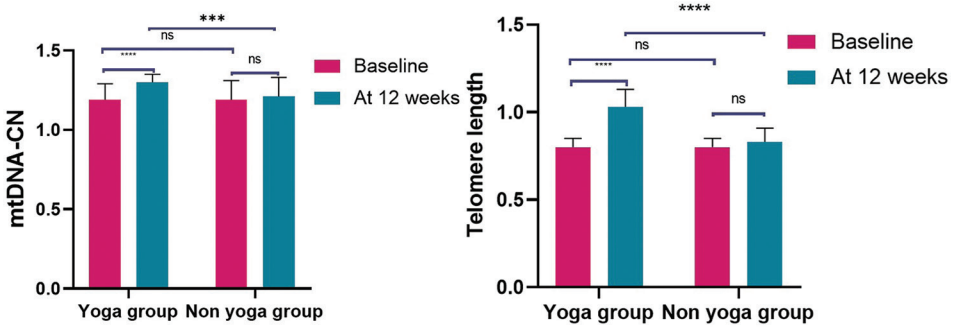

Figure 2: Analysis of mtDNA copy number and telomere length in the yoga and non-yoga group, data represented as mean ± standard deviation

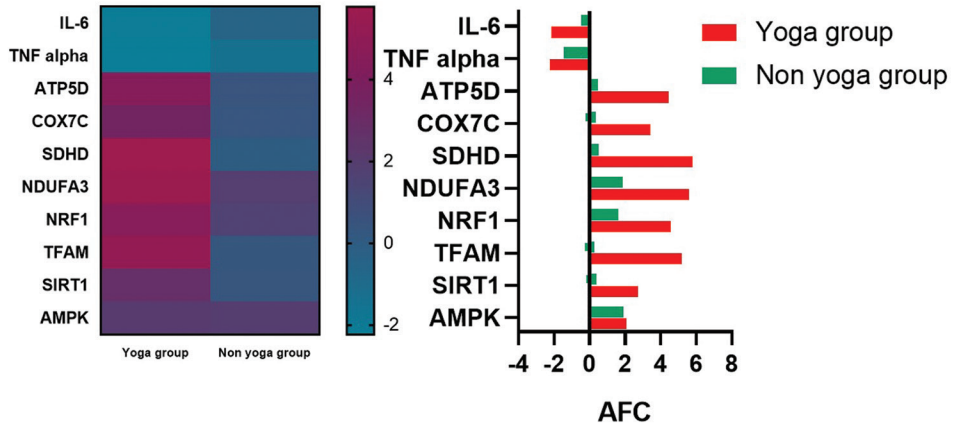

Figure 3: Relative mRNA expression mitochondrial integrity, mitochondrial respiratory chain, and inflammatory transcripts in the yoga and non-yoga group, AFC: Access fold change

post 12 weeks (between groups) [Figure 2]. In addition, NAD+ (−172.700, 95% CI [−308.200 to − 37.270]), and COX-II (−0.560, 95% CI [−0.861 to − 0.258]) levels showed a significant upregulation within the yoga group and post 12 weeks (between groups) [Table 4].

Secondary outcome

Depression severity

Significant reduction in the depression severity (6.000, 95% CI [3.411–8.589]) after 12 weeks of yoga practice and post 12 weeks (between groups) [Table 4].

Discussion

PCOS is a complex and prevalent neuroendocrine reproductive disorder. Early diagnosis and management are vital to prevent its progression and severity. To the best of our knowledge, this study highlights the beneficial effect of 12-week yoga practice on PCOS women. This study shows notable improvement in clinical, cellular, and molecular parameters. In the yoga group, we have observed the positive effects on PCOS women that are related to androgen homeostasis, OS, inflammation, and nuclear and mitochondrial genome.

Yoga and endocrine parameters

Following 12 weeks of yoga intervention, a significant increase in FSH levels, along with a significant decrease in LH, LH/FSH ratio, testosterone, and AMH levels are observed. Previous research suggested that adipocytes are prone to androgen overexposure resulting in obesity, OS, inflammation, IR, and compensatory hyperinsulinemia.[48] In addition, BMI reduction was also observed after yoga intervention. Hormonal balance and BMI reduction may contribute to OS optimization, inflammatory reduction, and improved mitochondrial functioning. All these improvements can potentially disrupt the vicious cycle associated with obesity, inflammation, mitochondrial dysfunctions, and OS.

Yoga and optimization of oxidative stress and inflammatory markers

OS and inflammation are associated with PCOS pathogenesis.[49] Follicular fluid, cumulus cells, and serum analysis of PCOS women revealed elevated levels of OS.[50,51] In this study, we have seen that in the yoga group significant reduction in the ROS, 8OHdG, and lipid peroxidation marker 4HNE levels while upregulation in TAC levels. TAC levels reduced in PCOS were directly associated with lower rates of oocyte maturation, fertilization, pregnancy rate, and poor embryo quality.[50,52] On the contrary, a positive relationship was observed between systematic antioxidant status and pregnancy success rates.[53] Furthermore, the lipid peroxidation profile

in the ovaries of PCOS patients is likely associated with inadequate progesterone production and elevated levels of LH.[54] Moreover, OS can activate NF-KB and JNK pathways, associated with inflammation and IR. In this study, we observed a reduction in the gene expression of *IL-6* and *TNF-α* after 12 weeks of yoga practice. Proinflammatory cytokine *TNF-α* alters the signaling of insulin through increased insulin receptor substrate-1 serine phosphorylation, subsequently leading to a reduction in the Glucose transporter type 4 gene expression.[55] This showed an anti-inflammatory effect which may enhance insulin sensitivity and glucose metabolism in the PCOS patient. The findings from our lab group suggested the positive effect of yoga on inflammatory cytokines and immune response.[56]

The mitochondrial genome is more prone to mutations than the nuclear genome because of the lack of a strong DNA repair mechanism and histone proteins and its closeness to ROS. As a result, mt-DNA damage is persistent and more severe in comparison to nuclear DNA.[57] Previous research has found significant elevated genomic instability and mitochondrial dysfunction in PCOS women.[58,59] Our laboratory data showed a decrease in genomic instability, as evinced by reduced levels of 8-OHdG, following the yoga-based lifestyle intervention.[60] Furthermore, telomere length preserves the nuclear genome stability. It controls the telomere metabolism and ensures cellular longevity. Women with PCOS have shorter telomere length as compared to healthy female with regular ovulatory cycle.[61] We have shown significantly higher levels of mtDNA-CN and telomere length in the yoga group than in the nonyoga group.

Yoga and mitochondrial activity

The yoga group shows improvement in mitochondrial parameters that contribute to energy requirements of high metabolic rate tissue. Furthermore, we have observed a significantly high level of nicotinamide adenine dinucleotide (NAD+). Lower NAD levels may lead to the dysfunction of NAD+-consuming enzymes, such as sirtuin. Their dysfunction alters mitochondrial functioning and intracellular signaling.[51] Various factors, including mitochondrial mutation and dysfunction, inflammation, and OS are involved in ovarian aging. In addition, NAD+ metabolism has a positive influence on oocyte, GCs, and embryo development which is crucial for ovarian aging. Hence, yoga may reverse ovarian aging through improvement in NAD+. NAD+ also improves cellular functions, through metabolic pathways, DNA repair, and chromatin remodeling.[62] In the yoga group, there was a rise in NAD+ levels and sirtuin. It may improve mitochondrial functioning. Another biomarker for aging is COX-II, which serves as the final enzyme in the MRC and plays a regulatory role in the mitochondrial OXPHOS system. Deficiencies in COX-II are associated with heightened OS and mitochondrial dysfunction.[63] Increased levels of COX-II induced by yoga regulate the cytochrome c oxidase super-complex and ultimately mitochondrial functioning.

PCOS women with different associated features such as IR exhibit compromised mitochondrial functioning.[64] MtDNA-CN significantly decreases in the peripheral blood of PCOS women.[65] Changes in mtDNA D-loop and alterations in copy number may represent an inheritable risk factor for PCOS.[14] Furthermore, HA has been shown to cause mitochondrial dysfunction in ovarian GCs in a rat model of PCOS. Additionally, these cells exhibited a lower mtDNA-CN that is linked to poor oocyte quality which can lead to compromised implantation and embryo development.[66,67] We have observed higher levels of leukocyte mtDNA-CN in the yoga group. Furthermore, yoga optimizes androgen levels, OS, and inflammatory markers, which are the key factors contributing to mitochondrial dysfunctions in PCOS. Mitochondrial health improvement may enhance oocyte quality and can improve implantation and embryo development outcomes.

Yoga and mitochondrial integrity

We have observed improved mtDNA integrity post-12 weeks of yoga intervention. It can be achieved through the elevated levels of mitochondrial transcripts associated with biogenesis and energy regulation. The specific transcripts that maintain mitochondrial integrity are *AMPK, SIRT-1, TFAM, and NRF1. AMPK* and *SIRT-1* are metabolic regulators and play important roles in lipid, glucose, and adipokine metabolism.[68] *SIRT1* is part of the sirtuin family of NAD+-dependent protein deacetylases. It regulates cellular metabolism, stress response, and longevity. Tao *et al*. showed significantly lower expression levels of both *AMPK* and *SIRT-1* not only in PCOS women's ovarian tissue but also in PCOS-induced rats.[69,70] Through the activation of many pathways that are dysregulated in PCOS patients, the AMPK-SIRT1 complex plays a critical role in maintaining the energy balance. Levels of *AMPK* and *SIRT1* increased after 12 weeks of yoga practice in PCOS patients. Hence, lifestyle modification like yoga can have multifaceted effects, addressing both the metabolic and reproductive aspects of PCOS. *NRF1* regulates the expression of transcripts involved in mitochondrial replication, transcription, and translation as well as nuclear-encoded OXPHOS.[71] Furthermore, it regulates *TFAM*, which initiates mtDNA transcription and replication. Altered levels of *NRF1* and *TFAM* can lead to mitochondrial dysfunction, impaired oxidative metabolism, and metabolic disorders. *TFAM* is lower in GCs of the PCOS group in comparison to the normal ovarian reserve control group.[72] Furthermore, studies have shown that whole ovarian tissue consistently shows lower levels of *TFAM* and *NRF-1*, which indicate defective mitochondrial biogenesis, despite using various methods to induce PCOS in rodents.[73-76] In addition, *NRF1* knockdown can reduce mtDNA-CN, *TFAM*

activity, ATP levels, and antioxidant enzyme levels. These events suggest its role in steroidogenesis, apoptosis, and OS regulation.[77] *NRF1* is involved in steroidogenesis, OS, and cell apoptosis. Its knockdown can significantly inhibit the expression of *steroidogenic acute regulatory protein* and *cytochrome P450 19A1*, leading to estrogen level reduction. Simultaneously, it suppresses the transcription and translation of superoxide dismutase, glutathione peroxidase, and catalase, decreased glutathione levels, and increased levels of 8-OHdG, indicating an impairment in OS regulation.[77] This study showed that the yoga group shows improved mitochondrial integrity (increased *NRF1* and *TFAM* gene expression), COXII activity, and MMP. It can restore steroidogenesis and cellular energy, and protect mtDNA. Yoga as a comprehensive approach may improve ovarian hormonal synthesis and reproductive outcomes.

Yoga and mitochondrial respiratory chain

Mitochondria are crucial for cellular energy production. Research suggests mitochondrial oxygen consumption declines in PCOS, whereas ROS production increases.[64] Skov *et al*. observed lower gene expression related to mitochondrial oxidative metabolism, including *NDUFA3, SDHD, Ubiquinol-Cytochrome c Reductase Core Protein, COX7C, and ATP Synthase Subunit H*, in the skeletal muscle of insulin-resistant PCOS women.[78] Moreover, lower maximal oxygen consumption (VO2 max) levels have been demonstrated in PCOS, with a strong correlation between OXPHOS transcript expression and VO2 max.[79,80] In this study, we have found a higher expression of OXPHOS transcripts in the yoga group. The findings of this study highlight that yoga improves mitochondrial oxidative metabolism and oxygen consumption, reduces OS and inflammation, and potentially reverses insulin resistance. Reduced inflammation is associated with increased expression of hepatocyte nuclear factor alpha a transcription factor regulating the synthesis of steroid hormone-binding globulin, thereby reducing free testosterone levels and thus reducing HA and its associated symptoms.

Yoga and comorbid depression

High-free testosterone levels in PCOS can cause hirsutism, acne, and androgenic alopecia that negatively impact self-image and lead to depression. Yoga practice reduces depression severity in PCOS patients. Additionally, our laboratory group has shown that yoga increases brain-derived neurotrophic factor and serotonin levels, improves neuroplasticity, and helps in managing major depressive disorder and comorbid depression.[40,60] Daily yoga practice integrated into our lifestyle reduces disease severity, lessens the severity of psychosomatic symptoms, and improves quality of life. Yoga can prevent, delay, and reduce PCOS severity by normalizing the hormone levels and improving physical, mental, reproductive health, and emotional well-being. As a result, it is a valuable adjunct therapy in the management of PCOS.

Our study has several strengths first, we have evaluated a wide range of parameters including clinical, cellular, and molecular with biomarkers such as mitochondrial transcripts, MMP, mtDNA-CN, and telomere length. Second, yoga integration can be a useful adjunct therapy in the management of PCOS. Regular practice can prevent the onset of other sequelae associated with this metabolic disorder. However, the study has limitations, including a small sample size, short intervention duration, and lack of blinding. Further, longer duration of practice is associated with poor compliance and a higher dropout rate. It is planned to study and follow-up on these cases after longer intervention and have active controls. We also plan to study the effect of yoga intervention on the complete transcriptome, metabolome, and lipidome to better understand the underlying mechanisms of yoga benefits.

Conclusions

PCOS is a complex lifestyle disorder that seems it affect ovaries but it is a systemic disorder. If it is not diagnosed and managed early can lead to systemic adverse effects such as NAFLD, CVD, hypertension, and Alzheimer's. There are antiandrogenic drugs, insulin-sensitizing agents and infertility management are mandatory for PCOS. However, it is a lifestyle disorder with a strong psychosomatic component, which needs to be managed by, lifestyle and mind-body intervention. Inflammation, OS, and mitochondrial dysfunction are significant underlying factors in its etiology. Yoga has the potential to tap the internal pharmacy of the body which improves mitochondrial integrity through the upregulation of transcripts related to mitochondrial integrity (*AMPK, SIRT 1, TFAM,* and *NRF1*) and MRC (*NDUFA3, SDHD, COX7C*, and *ATP5D*). In addition to mitochondrial integrity, it elevates mtDNA-CN, and telomere length, while reducing OS and inflammation. Yoga improves overall body functioning and is beneficial in managing symptoms and the severity of PCOS. Therefore, it serves as a rehabilitative approach to PCOS.

Ethical statement

The study was approved by the institutional Ethics Committee of AIIMS, New Delhi (IEC 606/15.07.2022).

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Conflicts of interest

There are no conflicts of interest.

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