Effect of Mindfulness Meditation on Intraocular Pressure and Trabecular Meshwork Gene Expression: A Randomized Controlled Trial

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• PURPOSE: To evaluate the effect of mindfulness meditation (MM) on intraocular pressure (IOP) and trabecular meshwork (TM) gene expression in patients with medically uncontrolled primary open angle glaucoma (POAG).

• DESIGN: Parallel arm, single-masked, randomized controlled trial.

• METHODS: Sixty POAG patients with IOP ≥21 mm Hg taking maximal topical medication and scheduled for trabeculectomy were included in this study at a tertiary eye care center in India. Thirty patients (Group 1) underwent 3 weeks of 45-minute daily MM sessions in addition to medical therapy while Group 2 continued medical therapy only. Primary outcome was change in IOP (ΔIOP) after 3 weeks of MM. Secondary outcomes were probability of success, percentage of reduction in IOP, effect on diurnal variations of IOP, changes in quality of life (QoL), and changes in gene expression patterns in TM.

• RESULTS: At 3 weeks, a significant decrease in IOP was seen in Group 1 (20.16 ± 3.3 to 15.05 ± 2.4mm Hg; $P = .001$), compared to Group 2 (21.2 ± 5.6 to 20.0 ± 5.8mm Hg; $P = .38$). ΔIOP was significantly higher in Group 1 than in Group 2 (5.0 ± 1.80 vs. 0.20 ± 3.03mm Hg; $P = .001$). Analysis of gene expression revealed significant upregulation of nitric oxide synthetase (*NOS1* and *NOS3*) and neuroprotective genes with downregulation of proinflammatory genes in Group 1 in comparison to Group 2 ($P = .001$).

• CONCLUSIONS: MM was associated with significant decrease in IOP and changes in TM gene expression, indicating its direct impact on ocular tissues. (Am J Ophthalmol 2021;223:308–321. © 2020 Elsevier Inc. All rights reserved.)

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THE MOMENT YOU CHANGE YOUR PERCEPTION IS the moment you rewrite the chemistry in your body.

—Bruce Lipton.[1]

Glaucoma is a neurodegenerative disease characterized by progressive loss of retinal ganglion cells (RGCs) leading to irreversible optic neuropathy.[2] Intraocular pressure (IOP) is a demonstrable and currently only modifiable risk factor associated with glaucoma progression.[3,4] Nevertheless, other mechanisms such as mitochondrial dysfunction and oxidative stress,[5] reduced nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) dysfunction,[6,7] inflammatory changes with glial activation, vascular dysregulation,[8] increased cortisol levels,[9] ischemia,[10] autonomic dysfunction,[11,12] neurodegeneration,[2] and glutamate excitotoxicity,[13] and others have also been shown to contribute to RGC death. Many of these factors can be accentuated by psychosocial or emotional stress.[14]

Mindfulness meditation (MM) is a set of contemplative practices aimed at balancing mental and emotional well-being by using mindfulness (conscious awareness) and directed attention. It is associated with a physiological state of reduced metabolic activity that enhances body balance and emotional stability, creating a relaxation response to counter the negative dimensions of stress response.[15] Using breath as an object of awareness, MM aims at achieving a mind-body harmony with holistic well-being. It has been shown to alleviate stress, anxiety, and depression and consequently improve mental and physical health.[16,17]

Glaucoma patients experience compromised quality of life (QoL) due to the psychological stress and co-morbid anxiety and depression-induced by the threat of vision loss, life-long treatment with significant economic burden, side effects of medication, hospital visits or stays, and so forth. These factors initiate a vicious cycle between stress-and-glaucoma severity. Stress can cause a rise in endogenous cortisol levels, which can in turn lead to increase in IOP. Recent studies have shown mind-body interventions are useful as an adjunctive therapy for glaucoma patients.[18] However the following important research questions remain: does meditation have a direct impact on ocular tissues, and what is the probable mechanism of IOP reduction with MM?

To answer these intriguing research questions, a randomized controlled trial (RCT) was conducted to evaluate the

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effects of MM on IOP and its impact on expression of the trabecular meshwork (TM) gene in patients with primary open angle glaucoma (POAG), whose POAG was not controlled by taking maximum tolerated topical medical therapy and were scheduled to undergo glaucoma filtering surgery (trabeculectomy).

METHODS

• **STUDY DESIGN:** A parallel arm randomized controlled trial (RCT) was conducted at the authors' tertiary eye care center (Dr. Rajendra Prasad Center for Ophthalmic Sciences, All India Institue of Medical Sciences, New Delhi, India). POAG patients from the authors' glaucoma service, meeting the eligibility criteria, were enrolled from November 2017 to January 2019. The study was approved by the Institute Ethics Committee (reference numbers IECPG-256/07.09.22017, RT-6/16.10.2017) and was prospectively registered with the clinical trial registry of India (Trial ID CTRI/2017/11/010534). This study was HIPAA compliant and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from each patient prior to enrolment. The study complied with the Consolidated Standards for Reporting Trials (CONSORT) protocol (Supplemental Figure).

• **PATIENT RECRUITMENT:** A total of 100 consecutive POAG patients who were being treated in the authors' glaucoma service and whose diagnoses were moderate to advanced POAG according to the Hodapp-Parish-Anderson classification[19] were screened for eligibility.

• **INCLUSION CRITERIA:** POAG patients >40 years of age with best corrected visual acuity >20/200 on Snellen's visual acuity chart, a chronically elevated IOP above 21 mm Hg on more than 3 occasions, taking maximum tolerated topical medical therapy, and optic nerve head (ONH) cupping greater than 0.7 (vertical cup-to-disc diameter ratio) and corresponding glaucomatous visual field changes were recruited. Patients had to be conversant in Hindi or English and be willing to attend a full course of MM.

• **EXCLUSION CRITERIA:** Patients with any other retinal or ON disorder causing perimetric defects, history of any angle-based surgery or laser treatment in the past, a history of any other ocular surgery within the past 12 months, a history of any psychiatric or psychological illness; comorbidities such as diabetes, coronary artery disease or cerebrovascular disease; and subnormal mentation and/or communication deficits were excluded. Those who were already practicing yoga and meditation in any form were also excluded from the study.

• **RANDOMIZATION:** Sixty of 100 patients meeting the eligibity criteria were assigned randomly to either intervention (Group 1) or control group (Group 2) with an allocation ratio of 1:1. This was done by a computer-generated randomizer using permuted blocks by the chief investigator (T.D.). The random sequence generated was transferred to an opaque, sealed envelope to conceal randomization until the actual allocation. Participants were enrolled by a co-investigator (J.S.), and participants were assigned to either group by a co-investigator (M.K.).

• **MASKING:** The investigators checking the IOP (N.B.) and studying gene expression (R.D.) were masked to the MM intervention.

• **STUDY PROTOCOL:** Baseline parameters included IOP by Goldmann applanation tonometry, best-corrected visual acuity using the Snellen chart, diurnal variation of IOP (DV^{IOP}), gonioscopy with Goldmann 2 mirror lens, stereoscopic ONH assessment with +90 diopter (D) lens and automated perimetry. Perimetry was performed using the 30-2 Swedish Interactive Threshold Algorithm standard program of the Humphrey field analyser (HFAII-i series; Zeiss Meditec, San Leandro, California, USA). No changes were made in the ongoing ocular hypotensive medications of the participants.

When a single value of IOP had to be studied, measurements were uniformly taken at 4 PM to avoid any transient effect of the meditative state on the IOP. For studying the diurnal variation of IOP, Goldmann applanation tonometry was carried out from 7 AM to 10 PM in 3-hour intervals by the same observer, and the range was noted down. Measurements were taken on the same tonometer attached to a slit-lamp biomicroscope each time.

Patients allocated to Group 1 underwent MM in addition to taking their routine medical therapy. They were enrolled for the sessions at the Integral Health Clinic of the authors' center between 10 AM and 11 AM daily for 21 days. Patients in Group 2 were advised to continue their medications as before and were scheduled for surgery after 3 weeks. At all times, the importance of continuing antiglaucoma medications and the role of meditation as an adjunct was reiterated.

• **OUTCOME AND SUCCESS CRITERIA:** The primary outcome was change in IOP (ΔIOP) after 3 weeks of MM. Secondary outcomes were probability of success (Defined as final IOP criteria A ≤15 mm Hg and >5 mm Hg, and criteria B ≤12 mm Hg and >5 mm Hg with ongoing ocular hypotensive medications after 3 weeks of MM), the percentage of reduction in IOP, the effect on diurnal variation of IOP, the change in QoL, and the changes in gene expression patterns in TM tissue.

• **REASSESSMENT:** The IOP and diurnal variations of IOP were reassessed in both groups by the same masked observer

at the end of 21 days. Patients who satisfied success criteria A in Group 1 were advised to continue the MM at home for 9 weeks. Patients who did not meet success criteria A in Group 1 and Group 2 underwent trabeculectomy with mitomycin-C by a single surgeon (T.D.). IOP was reassessed at 6 weeks after surgery (9 weeks from the time of enrolment) for all patients.

• **QUALITY OF LIFE ASSESSMENT:** Quality of life (QoL) was assessed using a glaucoma-specific quality questionnaire (GQL-15). There are 15 questions, and each question has a scale from 1 to 5 from less difficulty to severe difficulty to assess the degree of functional disability caused by glaucoma. A total score for the patient is calculated at the end of the study. Questionnaires in English and Hindi were distributed among patients on day 0 and at 3weeks.

• **TECHNIQUE OF MINDFULNESS MEDITATION:** MM was performed for 45 minutes under a certified yoga instructor. MM is simply observing the breath by focusing attention on the natural relaxed flow of air going in and of the body. Prior to this, the person sits in a chair with back and spine straight and erect. To be mindful is to be present in the moment. It is a state of awareness in a nonjudgmental way and heightens the person's patience, compassion, and kindness. Emphasis is on noticing the rise and fall of the chest and abdomen while inhaling and exhaling, respectively, and observing the sensation in the nostrils as cool air is drawn in and warm air is exhaled. The breath should be long and deep and the exhalation is kept longer than inhalation, and there is a pause after inhalation and exhalation. It is very easy to become distracted by thoughts, but thoughts are not resisted, rather, attention is refocused, drawn to the breath again.

• **SURGICAL TECHNIQUE FOR TRABECULECTOMY:** Patients were placed under peribulbar anaesthesia, and a corneal traction suture was placed using 6-0 polyglactin suture (Vicryl; Ethicon, Somerville, New Jersey, USA). A 6-8mm superotemporal fornix-based conjunctival flap was created, and hemostasis was achieved using wet field cautery. Cellulose acetate sponges soaked in 0.2 mg/mL mitomycin-C were applied subconjunctivally for 3 minutes. A partial thickness 5 × 5 mm scleral flap and 3 × 1 mm ostium was created. Excised 3 × 1 mm TM tissue was collected and transferred immediately to the molecular genetics laboratory for gene expression study. A wide peripheral iridectomy was performed. The scleral flap was closed with 2 10-0 nylon sutures, one at each corner. Peritomy was closed by wing-and-mattress sutures with 10-0 nylon. The standard postoperative regimen was given for all patients, which included topical antibiotic 4 times daily (moxifloxacin 0.5%), steroid 6 times daily (prednisolone acetate 1%), and mydriatic-cycloplegia 3 times daily (tropicamide 0.5%), tapered gradually over 8 weeks.

• **TRABECULAR MESHWORK ANALYSIS:** The procedure for obtaining the tissues was within the tenets of the Declaration of Helsinki. The removal of TM tissues from the anterior angle is a delicate procedure. Rigorous steps were thus followed to minimize contamination of the dissected TM by surrounding tissues. A portion of TM was processed for ultrastructural study to confirm the presence of TM. The remaining TM specimens obtained in the operating room were immediately placed in RNA Later (Ambion, Austin, Texas, USA). TM samples were incubated for 24 hours at room temperature to facilitate RNA Later (Ambion) permeation and then frozen at −80 C until further use. Total RNA from TM tissue was extracted using PicoPure RNA isolation kit (kit0204; Applied Biosystems, Foster City, California, USA) following the manufacturer's protocol.

• **RNA QUANTIFICATION AND CDNA CONVERSION:** The quality and quantity of RNA yield was determined using the NanoDrop (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and stored at −80°C until further use. RNA was treated with DNase I (Promega, Madison, Wisconsin, USA) and reverse-transcribed into complementary DNA (cDNA) using random hexamers and Revert Aid M-MuLv reverse transcriptase (Fermentas, Vilnius, Lithuania).

• **REAL-TIME POLYMERASE CHAIN REACTION AND GENE EXPRESSION ANALYSIS:** Genes were selected for expression analysis on the basis of previous microarray studies.[20] The selected genes were involved in many relevant cellular or molecular processes, for example, inflammation and glial activation, apoptosis, aqueous regulation, TM tissue maintenance, neuroprotection, neuroplasticity, oxidative stress, and trans-synaptic neurodegeneration.

Quantitative analysis of gene expression was performed (*IL4*, *MT1E*, *NGB*, *NGFR*, *NRG1*, *BCL2l11*, *EGFR*, *CARD8*, *MAPK10*, *MAPK15*, *NFkB1*, *TNFα*, *TGFβ*, *NOS1*, *NOS3*, *MNTR1A*, and *NR3C1*) by using CFX96 Real-Time System (Bio-Rad, Hercules, California, USA) using SsoFast EvaGreen Supermix (catalog no. 172-5200; Bio-Rad). Amplification reactions were performed in a 20-μL final volume containing 10 μL of SsoFast EvaGreen Supermix, 1 μL of primer, and 4 μL of cDNA. Amplification was carried out for 35 cycles for each gene. To normalize the amount of mRNA expressed, the internal housekeeping genes *β-actin* and *GAPDH* were used, and each cDNA product was tested in triplicate. Expression levels were (normalized to those of *β-actin* and *GADPH*) calculated as fold changes using the $2^{-\Delta\Delta C}{}_{t}$ method. Mechanism of action of these genes has been elaborated in Table 1.

The relative gene expression levels compared between controls and patients were assessed by comparing the mean fold changes between Group 1 and Group 2. Three steps were used to calculate the expression intensities: i)

TABLE 1. Genes Selected for Expression Analysis and Their Impact on Cellular Level

S.no	Gene Name	Details	Impact (Cellular Level)
1	NOS1 and 3	NOS1 and 3	• Reduction of actomyosin contractility and changes in the actin cytoskeleton (TM and inner wall of Schlemm's canal relaxation)[38] • Activation of large conductance calcium-activated potassium channel, involved in reducing TM cell volume[39] • Inhibition of *NFκB*, *iNOS*, *TNFα* (anti-inflammatory and antiapoptotic properties)[28] • Increased blood flow by vasodilation to the ONH[28,62]
2	MNTR1A	Melatonin receptor 1A	• Reduces oxidative stress (antiapoptotic)[45,46] • Decreases RGC death (neuroprotective)[45,46]
3	MT1E	Metallothionein 1E	• Reduces oxidative stress[63] • Decreases RGC death[63]
4	NGB	Neuroglobin	• Oxygen transporter[64,65] • Reduces oxidative stress and improves mitochondrial functioning[65] • Decreases RGC death[65]
5	*NGF* and *NGFR*	Nerve growth factor receptor	• Retinogenesis and RGC survival[66] • Neurotrophic and antiapoptotic effect[66]
6	NRG1	Neuregulin 1	• Neuroprotection[67]
7	NR3C1	Nuclear receptor subfamily 3 group C member 1	• Upregulation in expression of anti-inflammatory genes[47] • Decrease in endogenous cortisol level[47,48]
8	EGFR	Epidermal growth factor receptor	• Growth and thickening of TM band leads to reduction in pore size[58] • Neurodegeneration[59]
9	*TGFβ* and *IL4* and *NF-κB*	Transforming growth factor beta 1, interleukin 4 and nuclear factor kappa B	• TM fibrosis by increasing deposition of ECM proteins in TM cells[51,52] • Production of proinflammatory chemokines IL 8 and MCP 1 in TM cells[53–55] • Neurodegeneration[36,37]
10	*BCL2L11*	BCL2like 11	• Activation of BAX causes irreversible damage to mitochondria[68] • Ganglion cell death[69]
11	CARD8	Caspase recruitment domain family member 8	• Activation of caspases[70] • Facilitation of apoptosis and inflammation[70]
12	*TNFα*	Tumor necrosis factor	• Glial cell activation to produce neurotoxic substances[71] • Neurodegeneration and inflammation[72]
13	*MAPK10* and *MAPK15*	Mitogen-activated protein kinase 10 and 15	• Inflammation and apoptosis[73]

COX2 = cyclooxygenase 2; ECM = extracellular matrix; IL8 = interleukin 8; iNOS= inducible nitric oxide synthetase; MCP1 = monocyte chemoattractant protein-1; ONH = optic nerve head; PGE2 = prostaglandin E2; RGC = retinal ganglion cell; S.no = Serial number; TM = trabecular meshwork; TNFα/β = tumor necrosis factor alpha/beta.

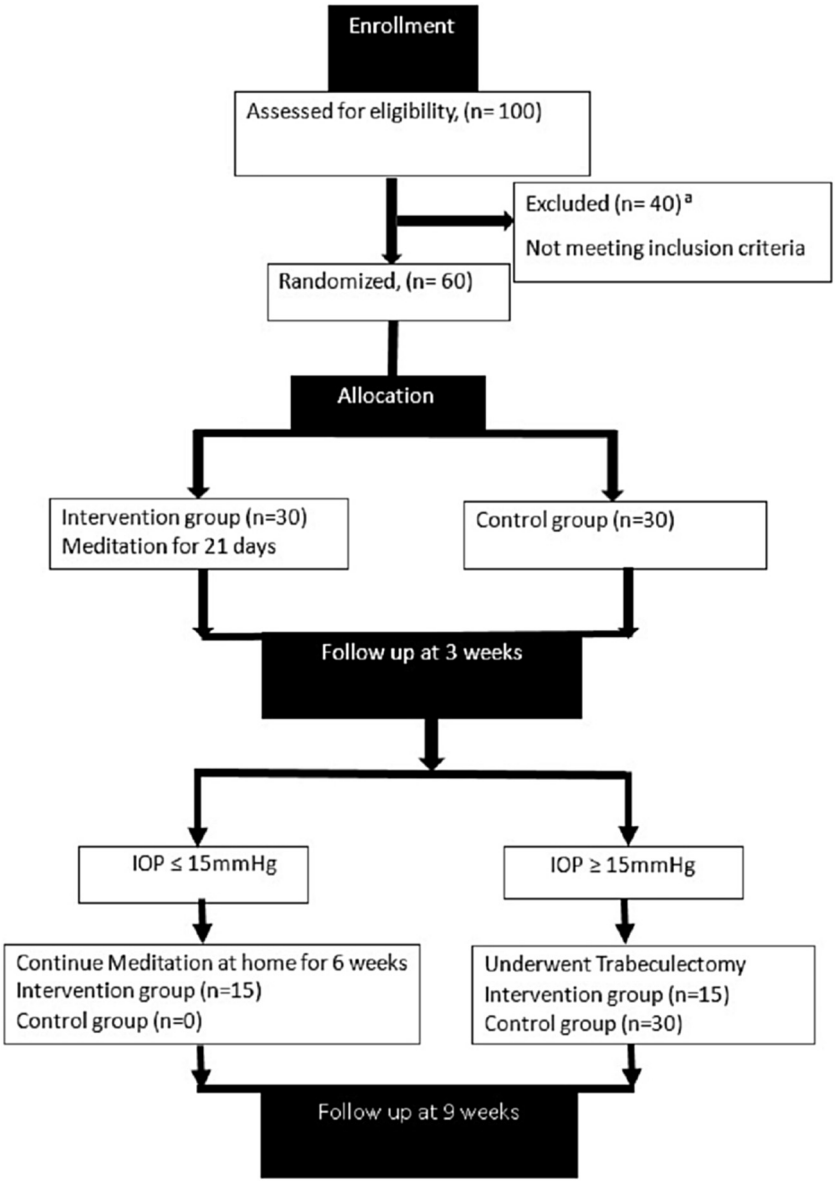

FIGURE 1. Overview of the study showing enrolment, randomization, group allocation, intervention, follow-up, and extended follow-up in the context of the study design. The study was a randomized controlled trial. (A) Twenty-nine patients refused to regularly attend the 3- week meditation course; 2 were already practicing meditation; 3 had a history of cataract surgery in the past 12; months and 6 had associated comorbidities

background correction; ii) normalization (by pairwise comparison followed by Benjamini and Hochberg false discovery rate correction; and iii) expression calculation. After estimation of mRNA expression intensity, a simple unpaired 2-tailed *t*-test ($P \leq .01$) was applied. The overall fold change of gene expression was calculated by the protocol described by Schmittgen and associates.[21]

• **DATA ANALYSIS AND STATISTICAL METHODS:** Because changes in IOP after MM have not been reported previously, absolute IOP values from the authors' previous studies were used to get an indicative sample size.[20] Sample size was computed to detect 15% reduction (from baseline value of 17.0 ± 2.7 to 14.45 ± 2.7 mm Hg) in IOP at 3 weeks post-enrolment with 95% confidence interval level and 90% power. The required sample size was determined as 24 per group. Considering a 20% loss to follow-up, a total of 60 eyes were enrolled in the study with 30 eyes in each group. One eye (the worst eye) was included for each patient.

Statistical analysis was performed using STATA version 12.1 software (STATA, College Station, Texas, USA). A paired *t*-test or Wilcoxon sign rank test was used to compare within group parameters. An independent *t*-test or Wilcoxon rank sum test was used to compare parameters

TABLE 2. Demography and Baseline Characteristics

Parameter	Group 1 Mean ± SD (n = 30)	Group 2 Mean ± SD (n = 30)	*P* Value[a]
Mean ± SD age, y	55.97 ± 8.98	55.79 ± 7.09	.92
Male: female ratio	23:7	22:8	.57
Mean ± SD baseline intraocular pressure, mm Hg	20.16 ± 3.3	21.29 ± 5.65	.26
Mean ± SD cup-to-disc ratio	0.86 ± 0.05	0.88 ± 0.04	.07
Mean ± SD deviation, dB (Humphrey field analyzer)	−14.15 ± 3.65	−15.2 ± 2.92	.19
Mean ± SD best-corrected visual acuity (logMAR[b])	0.408 ± 0.535	0.413 ± 0.463	.96
Mean ± SD preoperative topical ocular hypotensive medications	3.58 ± 0.87	3.20 ± 1.21	.187
Mean ± SD diurnal variation fluctuation	4.07 ± 1.3	4.5 ± 2.17	.355
Mean ± SD GQL-15[c] score	64.08 ± 4.56	65.66 ± 5.65	.217

[a]Paired *t* test; *P* value <0.05 is significant.
[b]logMAR = logarithm of the minimum angle of resolution.
[c]GQL-15 = glaucoma quality of life-15 questionnaire.

TABLE 3. Comparison of Changes in IOP at 3 Weeks

Parameter: IOP, mm Hg[a]	Group 1 (n = 30)	Group 2 (n = 30)	*P* Value[b]
Mean ± SD baseline	20.16 ± 3.3	21.29 ± 5.65	.371
Mean ± SD at 3 weeks	15.05 ± 2.49	20.05 ± 5.85	.001
Mean ± SD change in IOP	5.0 ± 1.80	0.20 ± 3.03	.001

[a]IOP = intraocular pressure.
[b]Paired *t* test; *P* value <0.05 is significant.

between the 2 groups and an analysis of variance (ANOVA) or Kruskal-Wallis test for comparing more than 2 variables. To address multiplicity, a Bonferroni correction was applied wherever required. A *P* value of <.05 was considered statistically significant.

• **RESULTS:** A total of 60 patients were recruited (30 in each group) after excluding the ones who were not eligible (Figure 1). Of 40 patients who were ineligible, 29 patients refused to regularly attend the 3 week MM course, 2 were already practicing meditation, 3 had a history of cataract surgery in the past 12 months, and 6 had associated comorbidities. Baseline clinical characteristics were comparable in both groups (Table 2). There were no significant differences between the class of glaucoma medications being used in the 2 groups, prostaglandin analogues ($P = .37$); beta-blockers ($P = .21$); alpha-agonists ($P = .34$); and topical carbonic anhydrase inhibitors ($P = .77$).

• **AT 3 WEEKS' REVIEW:** *Change in IOP.* The mean baseline IOP was 20.16 ± 3.3 mm Hg in Group 1 and 21.29 ± 5.65 mm Hg in Group 2 ($P = .26$). A significant decrease in IOP from the baseline (20.16 ± 3.3 to 15.05 ± 2.49; $P = .001$) was seen at 3 weeks in Group 1 compared to Group 2 (21.29 ± 5.65 to 20.05 ± 5.85; $P = .38$). ΔIOP was significantly greater in Group 1 (5.0 ± 1.80) than in Group 2 (0.20 ± 3.03; $P = .001$. (Table 3).

Percentage of reduction in IOP. The percentage of reductions of IOP at day 21 with respect to their baseline IOPs were calculated and categorized. The mean percentage of reductions in IOP was 23.34% in Group 1 ($P = .01$) and 5.82% in Group 2 ($P = .38$) at 3 weeks. Up to 86% of patients in Group 1 showed >15% reduction in the IOP, among whom 33.33% showed >25% reduction (Figure 2).

Success rate. Success was obtained in 15 of 30 cases (50%) in Group 1 at 3 weeks, whereas none of the controls achieved success according to criteria A ($P = .009$). For criteria B (IOP ≤ 12 mm Hg), success was achieved in 7 of 30 patients (23.3%) in Group 1 and none in Group 2 ($P = .028$) (Figure 3,A).

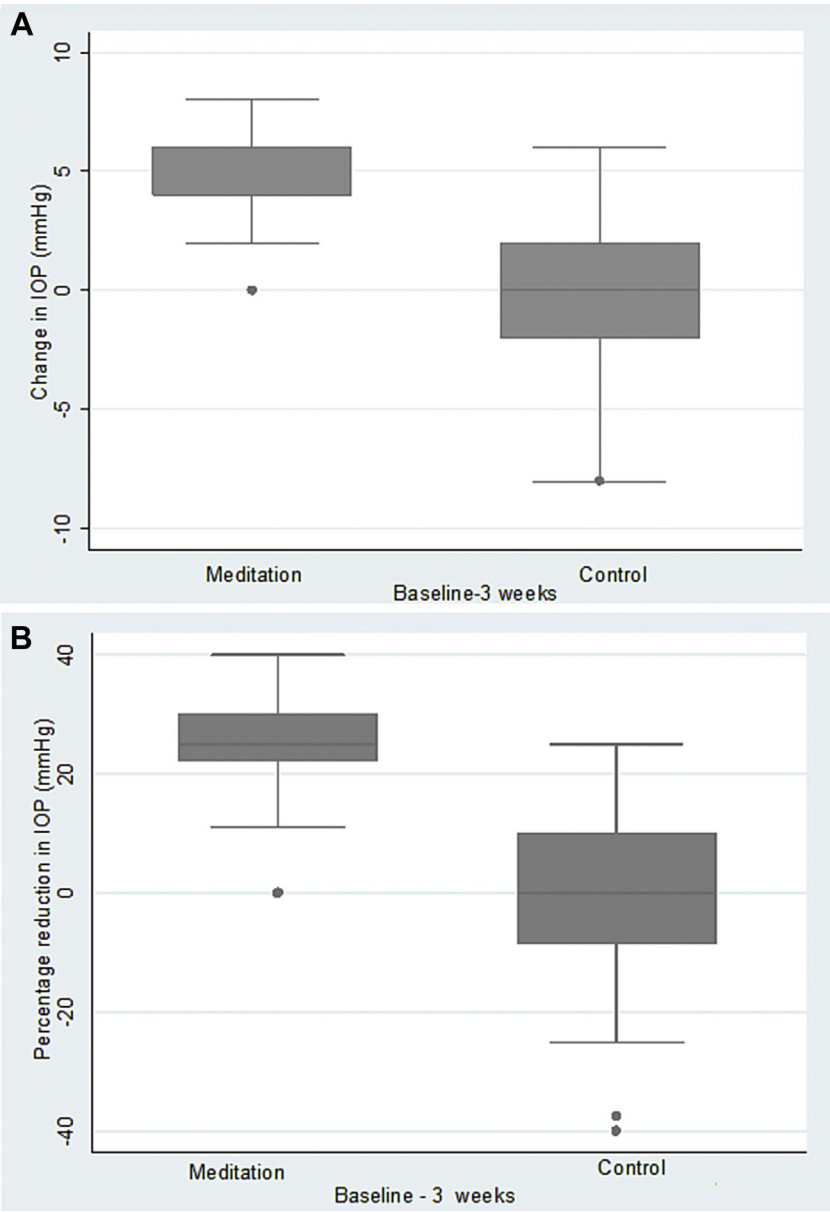

FIGURE 2. (A) Box plot shows change in IOP at 3 weeks in Group 1 (meditation) and Group 2 (control). (B) Box plot shows percentage reduction in intraocular pressure from baseline in Group 1 (meditation) and Group 2 (control) at 3 weeks. Error bars are the 95% confidence interval; the bottom and top of the box are 25th and 75th percentiles, respectively. The line inside the box is the 50th percentile.

Diurnal Fluctuation of IOP. The mean fluctuations in DV^{IOP} were comparable between the 2 groups at baseline (Table 2). In Group 1, a significant decrease in DV^{IOP} (4.07 ± 1.3 to 2.95 ± 1.75 mm Hg) was seen at 3 weeks ($P = .006$) in comparison to Group 2 (4.5 ± 2.17 to 4.38 ± 2.3 mm Hg; $P = .83$). There were significant differences in DV^{IOP} at 3 weeks between the 2 groups ($P = .001$).

Change in quality of life. The GQL-15 questionnaire scores of both groups were comparable at recruitment ($P = .217$). After 3 weeks, there was a significant decrease in the GQL-15 score in Group 1 (64.08 ± 4.56 to 40.05 ± 5.11; $P = .0001$) in comparison to that in Group 2 (65.66 ± 5.65 to 64.23 ± 4.43; $P = .661$), pointing toward improvement in the quality of life in glaucoma patients after 3 weeks of meditation.

• **AT 9 WEEKS' REVIEW:** *Change in IOP from baseline*. Patients who achieved success in Group 1 and continued MM at home (group: meditation-no surgery) along with medical treatment, maintained IOP at

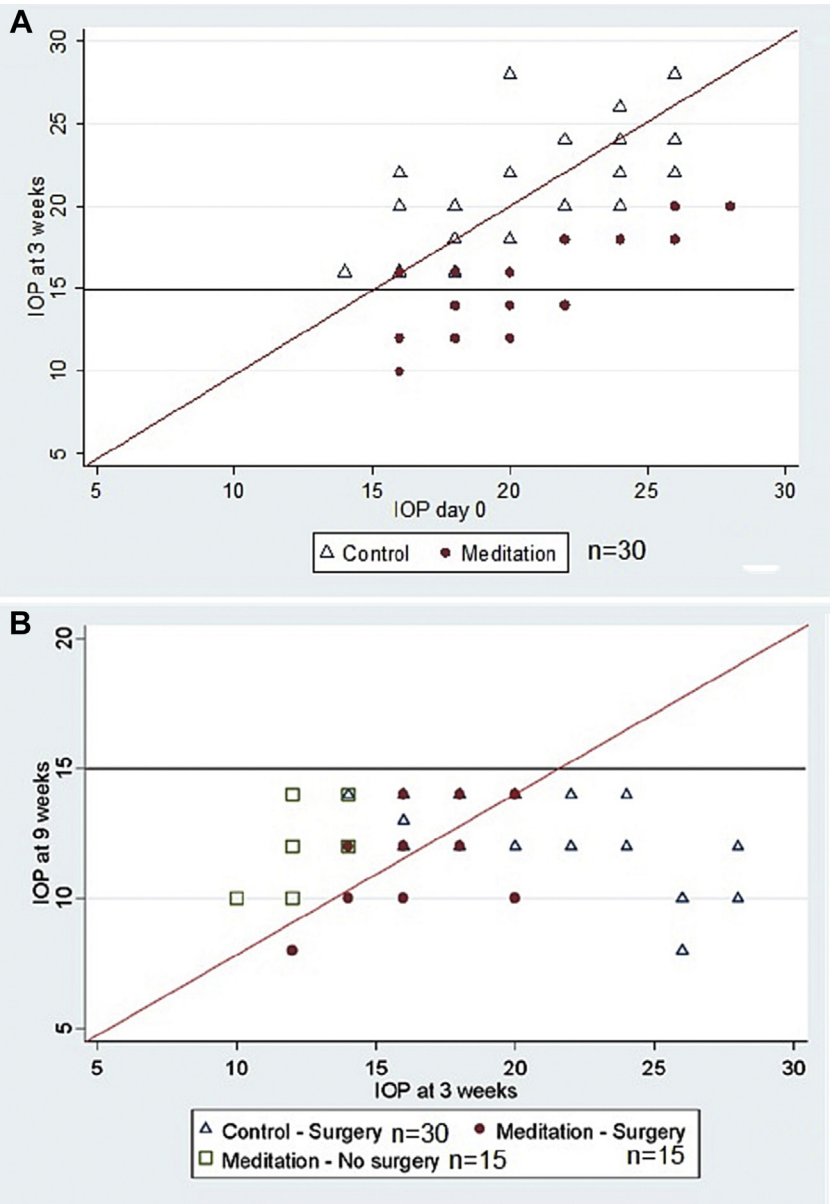

FIGURE 3. (A) Scattergram shows IOP distribution of Group 1 (meditation) and Group 2 (control) at 3 weeks from baseline. (B) Scattergram shows intraocular pressure distribution of Group 1 (meditation → no surgery and meditation → surgery) and Group 2 (control) at 9 weeks. Cutoff lines define IOP < 15 mm Hg as success. IOP = intraocular pressure.

9 weeks (12.8 ± 1.47 mm Hg; ΔIOP: 5.44 ± 1.96 mm Hg; $P = .0001$). Mean IOP of patients in Group 1 who underwent trabeculectomy (group: meditation-surgery) was 11.78 ± 1.83 mm Hg (ΔIOP: 9.56 ± 3.56 mm Hg; $P = .0001$), and Group 2 was 13.25 ± 2.92 mm Hg (ΔIOP: 7.70 ± 3.83 mm Hg; $P = .0001$). The mean IOP was comparable in the above 3 groups ($P = .1145$). ΔIOP was also comparable among groups, except a significantly higher change in IOP was observed in the meditation-surgery group versus the meditation-no surgery group ($P = .0005$) (meditation-surgery vs. control: $P = .130$; meditation-no surgery vs. control: $P = .063$).

Success rate. At 9 weeks (6 weeks post-trabeculectomy) both groups achieved 100% success according to criteria A ($P = .85$) (Figure 3B). According to criteria B, a 73% success rate was observed in Group 1 (n = 15) versus 53% success rate in Group 2 (n = 30) at 6 weeks post-trabeculectomy. This result was statistically significant ($P = .01$), suggesting better outcomes in trabeculectomy post-MM. None of the patients in either group required suture lysis.

Trabecular meshwork gene expression. Gene expression analysis showed a significant upregulation of the following genes: *NGB*, *NGFR*, *NRG1*, *NOS1*, *NOS3*, *MNTR1A*,

TABLE 4. Fold Change of Gene Expression in Trabecular Meshwork: Group 1 Compared to Group 2

S. No.	Gene Name	Details	Primer Sequence	Fold Change (Trabecular Meshwork)	*P* Value[a]
Upregulated Genes					
1	NOS1	Nitric oxide synthase 1	CCCCACCTCAGGAAAACAGTC CTCTGTGTCCTTGAGCTGGTAAG	6.092	.001
2	NOS3	Nitric oxide synthase 3	TGATGGCGAAGCGAGTGAAG ACTCATCCATACACAGGACCC	2.497	.001
3	NR3C1	Nuclear receptor subfamily 3 group C member 1	ACAGCATCCCTTTCTCAACAG GATCCTTGGCACCTATTCCAAT	3.193	.001
4	MNTR1A	Melatonin receptor 1A	CTGTCGGTGTATCGGAACAAG CCAACGGGTACGGATAAATGG	25.551	.001
5	NGB	Neuroglobin	AAGGTGATGCTCGTGATTGATG CCCGTAGAGTTGGCTCCAG	5.943	.001
6	NRG1	Neuregulin 1	GGAGCTGTACCAGAAGAGAGTGC CAGTAGTGGAGTGATGGGCTGTG	14.340	.001
7	NGFR	Nerve growth factor receptor	GGGGTAGACCTTGTGATCC CTCAGATGAAGCCAACCACG	4.515	.001
8	MT1E	Metallothionein 1E	AGAAGAGTGAGTGCGGG AGGTTGTGCAGGTTGTTC	2.63	.001
Downregulated Genes					
1	TNFα	Tumor necrosis factor	ACCTCCGAGATGACACCATCA GGCACTCTGGCACATATTCAC	−2.692	.001
2	TGFβ	Transforming growth factor beta 1	GAAGGGAGACAATCGCTTTAGC TGTAGACTCCTTCCCGGTTGAG	−6.067	.001
3	IL4	Interleukin 4	AAATATGCGAAGCACCTTGG TTGAACGAGGTCACAGGAGA	−8.632	.001
4	BCL2L11	BCL2 like 11	GTTGAACTCGTCTCCGATCC GCCCCTACCTCCCTACAGAC	−2.361	.001
5	CARD8	Caspase recruitment domain family member 8	AGCAGGGTTTGTCTGGGC CAGTGTGCCGATGCTGCC	−47.365	.001
6	MAPK10	Mitogen-activated protein kinase 10	CGCTGCTTCCTCTACCCC CTGTGACTATGACTACCC	−6.815	.001
7	MAPK15	Mitogen-activated protein kinase 15	CGTCGGTTCCCACGGCAA GGGCATCTGTCTTATCCC	−18.668	.001
8	NFkBA1	Nuclear factor kappa B subunit 1	GAAGAAGGAGCGGCTACT CTCGAAAGTCTCGGAGCT	−8.070	.001
9	EGFR	Epidermal growth factor receptor	AGGTTGTAAGGGGGAGCA CCAGTGCCTTTCCTGCTAA	−14.875	.001
10	FGFR1	Fibroblast growth factor receptor 1	AGACCCCTCGTAGCGCAT GTTCCGCCTCGGGAGAGT	−11.256	.001

S.No. = Serial number
[a]Paired *t* test, *P* value <.05 is significant.

NR3C1, and *MT1E* and a significant downregulation of *IL4*, *BCL2l11*, *CARD8*, *FGFR1*, *MAPK10*, *MAPK15*, *TGFβ*, *TNFα*, *NFkB*, and *EGFR* in Group 1 in comparison to Group 2 (Table 4, Figure 4).

Complications. No complications or side effects were observed due to mindfulness meditation. No intraoperative complications were observed in either group. In the immediate postoperative period, shallow anterior chamber was noted in 1 eye in Group 1 and in 3 eyes in Group 2. All 4 patients were managed conservatively.

DISCUSSION

MEDITATION IS A UNIQUE INTERVENTION BECAUSE IT TARgets the brain and impacts multiple mechanisms of

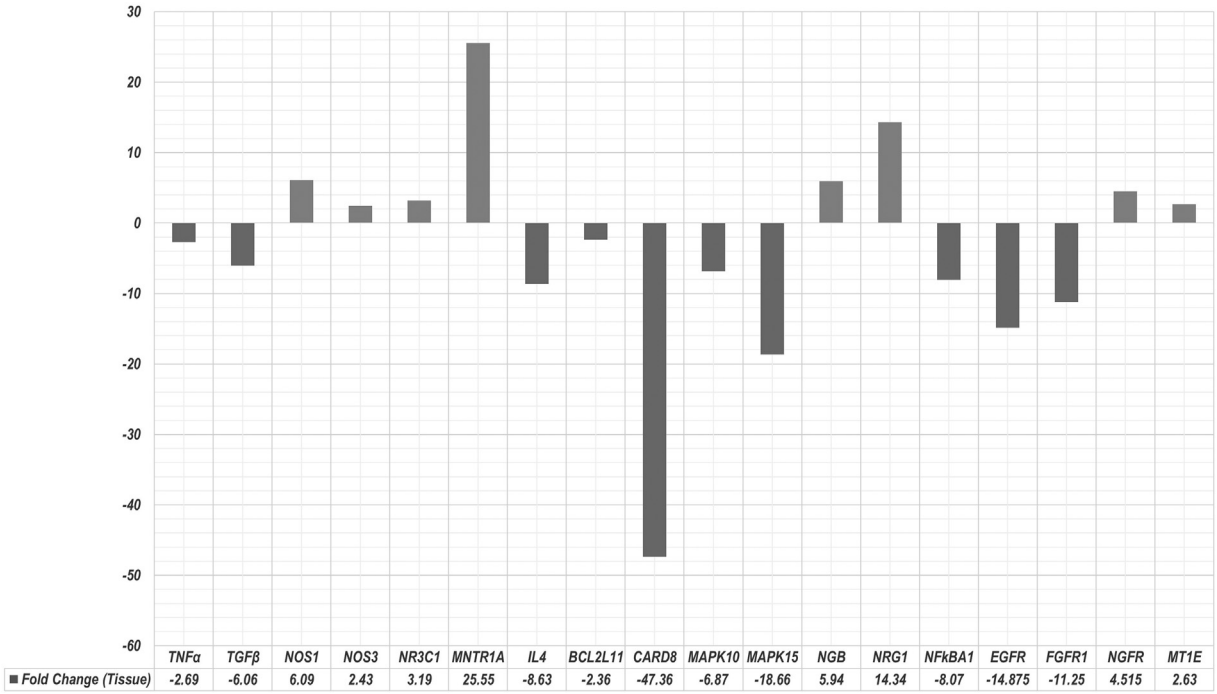

FIGURE 4. Diagram shows the fold change of gene expression in the trabecular meshwork of Group 1 compared to that in Group 2.

glaucoma pathogenesis, thereby having the potential to serve as a "polypill" for comprehensive glaucoma management.[18] Glaucoma is increasingly recognized as a "sick eye in a sick body" syndrome because it has been associated with abnormalities in cardiovascular, central nervous system, endocrine, and psychological systems, which can be further accentuated by stress, and there is a paramount need for the ophthalmologists and health care providers to understand the utility of stress reduction therapies for glaucoma patients.[22,23]

MM can modify the course of glaucoma by several mechanisms: direct effect on IOP,[20] decreasing endogenous cortisol,[20] decreased inflammation and oxidative stress,[20] improved blood flow, with oxygenation of the brain,[24,25] upregulation of neurotrophins such as BDNF,[23] and direct impact on central and autonomic nervous sysytems.[26,27]

The decrease in IOP can be attributed to 1) an increase in nitric oxide-mediated trabecular outflow[28]; 2) an increase in melatonin levels, which negatively impacts aqueous production[29,30]; 3) a decrease in endogenous cortisol[19,30]; or 4) the impact on the parasympathetic nervous system and central nervous system.[27]

In an RCT conducted by Dada and associates,[20] a 3-week course of MM in POAG patients resulted in significant lowering of IOP and changes in systemic gene expression. Upregulation of anti-inflammatory genes and downregulation of pro-inflammatory genes were studied using RNA extracted from whole blood after the patients completed the course of MM. Gagrani and associates[24] also reported lowering of IOP, increased oxygenation of the brain, improved QoL, and reduced levels of serum cortisol in POAG patients after 6 weeks of MM. The current study also showed consistent results. A significantly mean lowering of IOP (23%) was observed which obviated the need for glaucoma filtering surgery in 50% of the patients.

Studies have shown that with every 1 mm Hg[32] drop in IOP, the risk of glaucoma progression is reduced by 10%. Taking into account the above numbers, the results of this study with an IOP drop of nearly 5 mm Hg translates to an enormous impact on the course of the disease. This is particularly important for a disease such as glaucoma that has high prevalence, is progressive in nature, and exerts huge economic burden on the society.

We also reported for the first time a significant decrease in diurnal fluctuation of IOP in the MM group. The measurement of IOP (phasing) over a 24-hour period is important to ensure that the peaks in IOP (if any) are not missed in apparently well-controlled glaucoma patients.[33] In a prospective study, Asrani and associates[33] followed patients with OAG who were monitored by visual field and home tonometry 5 times daily. Patients in the highest quartile of home IOP range (at least 11.8 mm Hg) had a cumulative risk of visual field progression of 88% over 8 years, compared to 57% for patients in the lowest quartile for IOP range (no more than 7.7mm Hg). Large fluctuations in diurnal IOP have been verified and shown to be independent risk factors[34] for glaucoma progression, even in eyes with reasonably controlled office IOPs over years. Shukla and associates[35] showed that the effect of IOP fluctuation on rate of visual field progression was equivalent to mean and maximum IOP values, emphasizing the fact that

diurnal IOP fluctuation is an important consideration in management of glaucoma patients.

Downregulation of the proinflammatory genes and upregulation of the anti-inflammatory elements were observed in Group 1. We found a significant expression upregulation of *NOS1*, *NOS3*, *MNTR1A*, *NGB*, *NRG1*, *NGFR*, *NR3C1*, *MT1E* and a significant downregulation of *TGFβ*, *IL4*, *TNFα*, *BCL2L11*, *NFKβ*, *CARD8*, *EGFR*, *MAPK10*, and *MAPK15*. These changes consequently decreased IOP, reduced neuroinflammation, and prevented RGC death.[20,24,36] Creswell and associates[37] demonstrated a postmeditative decrease in proinflammatory C-reactive protein and *NFκB*-related gene expression in circulating leucocytes. In a previously published study, the effect of MM on gene expression in POAG patients was analyzed after extracting total RNA from whole blood.[20] The current study is the first to report changes in gene expression in RNA extracted directly from TM. This study unravels the avant-garde concept of direct impact of meditation on ocular tissues and its salutary influence on IOP and RGCs.

The gene expression modulation in trabecular meshwork and its implications are discussed below and in Tables 1 and 4.

Upregulation of both endothelial and neuronal nitric oxide synthetase (NOS3 and NOS1) was seen in glaucoma patients who underwent a 3-week meditation course in this study. The anatomical distribution of NOS plays a key role in modulating aqueous outflow at the level of TM, Schlemm's canal, and collecting channels, or indirectly through alteration in the tone of the longitudinal ciliary muscles.[38] This upregulation causes increases in NO levels, leading to activation of large conductance calcium-activated potassium channels, involved in reducing TM cell volume.[39] NO donors may trigger and cause reduction of actomyosin contractility, changes in the actin cytoskeleton, changes in the cell adhesion system, and cause overall relaxation of the TM and inner wall of Schlemm's canal, leading to decreased resistance to aqueous humor outflow.[40] The commercially available ocular hypotensive agent latanoprostene bunod 0.024% has butanediol mononitrate as 1 of its active metabolites, which is an NO donor and works on the same principle as mentioned earlier.[40,41] Pang and associates[42] documented the fact that NO is important for modulating the dynamic balance between the rate of secretion (inflow) and drainage (outflow) of aqueous humor. NO also inhibits NFkB and proinflammatory proteins such as iNOS and TNF, and thus has anti-inflammatory and antiapoptotic properties. Cavet and associates[28] also documented the fact that elevated *NOS3* increased blood flow by vasodilation to the ONH and thus was neuroprotective. NO also regulates basal ocular vascular tone and thus may have positive effects on cell health and health of ocular tissues.[43] Upregulation of endothelial NOS3 with increases in trabecular outflow can be an important pathway, explaining the direct impact of MM on IOP. Also, the multiple systemic beneficial effects reported after the "relaxation response" are mediated by nitric oxide.

Increased expression of the melatonin receptor (*MNTR1A*) was seen in the intervention group of this study. Meditation has been proven to produce higher plasma levels of melatonin through direct stimulation of the pineal gland.[44] In a pilot study by Pescosolido and associates,[30] POAG patients with uncontrolled IOP, even taking maximal topical medications, showed a significant reduction in IOP with oral agomelatonin (an oral melatonin analog) compared to the controls.[30] Alkozi and associates[29] also described the significant role of melatonin in the maintenance of pressure homeostasis in both normotensive and hypertensive eyes. Thus, upregulation of the melatonin receptor could be a major mechanism for IOP reduction. Additionally, melatonin has neuroprotective and antiapoptotic role, reducing oxidative stress and RGC death.[45,46]

An increased expression of glucocorticoid receptor gene (*NR3C1*) was seen post-meditation, which is postulated to reduce overall cortisol levels (higher glucocorticoid receptor sensitivity relays the same anti-inflammatory effects at lower doses of circulating cortisol).[47,48] Stress reduction conferred by meditation causes decline in corticotropin-releasing hormone, cortisol, and catecholamine levels. These endogenous steroids have been directly related to increases in IOP under certain conditions.[49] In the RCT conducted by Dada and associates,[19] the meditation group had a significant lowering of IOP compared to controls (75% of patients in the intervention group had more than 25% reduction in IOP), which was directly correlated with a decrease in serum cortisol.[20] MM is hypothesized to cause activation of transrepressor isoform of the glucocorticoid receptor GR-beta (GRB). GRB causes most of the beneficial effects by inhibiting *TGFβ* and is thus protective in glaucoma.[50] Thus, meditation decreases serum cortisol and regulates stress response leading to a healthy homeostasis, thereby, lowering IOP and neuroinflammation.[31]

In glaucomatous eyes, increased *TGFβ2* activity causes TM fibrosis by increasing the production and deposition of extracellular matrix proteins in TM cells, thereby blocking the aqueous humor outflow.[51,52] *IL4* and *TGFβ* regulate production of the proinflammatory chemokines IL-8 and MCP1 in TM cells, and their levels are elevated in glaucoma.[53-55] Expressions of *TGFβ* and *IL4* showed a significant decrease in Group 1. *TGFβ* also stimulates conjunctival fibroblast function and is released during trabeculectomy from platelets, neutrophils, macrophages, and fibroblasts at the site of injury.[56] *TGFβ* then stimulates formation of granulation tissue. Ng and associates[57] demonstrated that lower levels of conjunctival *TGFβ* were associated with a higher success rate of trabeculectomy, potentially due to reduced scarring in the postoperative period. In the current study, 6 weeks post-trabeculectomy, no significant differences were seen in mean IOP of the 2 groups. However, success according to

criteria B was achieved in 73% of cases in Group 1 versus only 53% in Group 2, which may be attributed to the anti-inflammatory effect of MM on ocular tissues. Studies with longer duration of follow-up will further elucidate this difference between the two groups.

Epidermal growth factor binds to *EGFR*s on the membrane of TM cells which causes thickening of TM bands and thus decreased pore size and offers resistance to aqueous outflow.[58] Downregulation of *EGFR* was observed in Group 1, and this may contribute to enhancing aqueous outflow and thus decreasing the IOP. Upregulation and activation of *EGFR* is one of the key factors of neurodegenerative diseases,[59] and its inhibition can prevent glaucomatous injury through loss of RGCs.

Although the impact of MM on TM gene expression and intraocular pressure is still neoteric, its potential for reducing psychological stress has been known for a protracted period. In concordance with the authors' previous studies, significant improvement in quality of life was noted in the intervention group compared to the control group.[20,24] Patients with glaucoma are subjected to psychological stresses such as the burden of being on multiple medications, ocular surface disorders, functional disability of varying extent, long-term follow-up, and hospital visits. Decrease in the GQL-15 score in the intervention group indicated the effect of MM on mental well-being and on other domains demanding functional peripheral vision, dark adaptation, glare, outdoor mobility, central and near vision.[20,24]

On continuing MM, the effect was seen to be sustained at 9 weeks of observation. Up to 86% of participants had at least 15% reduction in IOP, which is nearly equivalent to the effect of 1 antiglaucoma medication. It is worthwhile to mention that an ideal[60] anti-glaucoma medication is the one which effectively lowers IOP, produces no local or systemic adverse effects, is inexpensive, and has once-a-day dosing. Meditation is now emerging as a novel method for IOP reduction with added benefits of neuroprotection, decreased psychological stress, holistic health benefits, and a resultant improvement in quality of life. None of the current glaucoma therapies offers the dual benefit of lowering IOP and simultaneously improving quality of life without a financial burden on the patient, and this highlights MM as a unique intervention that should be added in the armamentarium of mainstream glaucoma management.

Last but not least, the impact of meditation on altering patient's perception toward this lifelong chronic disease cannot be overlooked. The epigenetic evidence suggests that environmental factors (nutrition, stress, thoughts, and so forth) control the binding of regulatory proteins to DNA and thus modify gene expression. Changes in perception or stress reduction due to meditation can potentially alter the cell biology and positively regulate TM gene expression.[61]

• **STUDY LIMITATIONS:** One of the major limitations of this study is the small sample size with a short duration of intervention and observation. The 3-week MM training was conducted under a trained instructor; however, compliance and replication of the same at home on a long-term basis in the absence of expert supervision is uncertain. Significant improvement in GQL-15 scores in the intervention group also needs cautious interpretation, as 3 weeks is a relatively short duration to assess the impact on QoL. Because the patients were under daily observation in the intervention group, the bias introduced by the Hawthorne effect cannot be excluded.

CONCLUSIONS

MM WAS EFFECTIVE IN CAUSING A SIGNIFICANT LOWERING of IOP in POAG patients with a concomitant reduction in diurnal fluctuation of IOP. When added as an adjunct to the routine glaucoma medications, filtration surgery could be avoided in up to 50% of patients in the present study. Eyes that underwent trabeculectomy after the MM course also had better outcomes in the early postoperative period than those in the control group. Alterations in gene expression revealed that MM positively modulated cellular mechanisms involved in glaucoma pathogenesis (through both IOP-dependent and IOP-independent mechanisms).

Uncited References

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