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Circulating microRNAs as Biomarkers for Primary Open-Angle Glaucoma: A Case-Control Study

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Research Article

Keywords:

Posted Date: February 24th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8814911/v1

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Additional Declarations: No competing interests reported.

Abstract

Background

Primary open-angle glaucoma (POAG) is a leading cause of irreversible blindness worldwide. Early diagnosis remains challenging, highlighting the need for reliable biomarkers. In recent years, circulating microRNAs (miRNAs) have emerged as potential minimally invasive biomarkers in ocular diseases.

Objective

This study aimed to evaluate the expression of two selected miRNAs i.e, miR-210-3p and miR-143-3p, in plasma and aqueous humor (AH) of POAG patients, cataract patients, and healthy controls, and to assess their diagnostic potential.

Methods

Plasma and AH samples were collected from 30 POAG patients, 30 cataract patients, and 30 healthy controls. Small RNAs were isolated, and expression of miR-210-3p and miR-143-3p was quantified by TaqMan™ qPCR assays using miR-16-5p as an endogenous control and cel-miR-39 as an exogenous control. Data was analyzed using the $2^{\wedge -\Delta\Delta Ct}$ method. Diagnostic accuracy was assessed by receiver operating characteristic (ROC) analysis.

Results

miR-210-3p expression was significantly elevated in plasma (median fold change 5.18, $p < 0.0001$) and AH (median fold change 2.81, $p = 0.0003$) of POAG patients compared with cataract and normal controls. Plasma ROC analysis for miR-210-3p yielded an AUC of 0.862 (95% CI: 0.751–0.936), with 86.2% sensitivity and 87.9% specificity. In contrast, miR-143-3p was significantly upregulated only in AH of POAG patients (median fold change 8.24, $p < 0.0001$), but not in plasma. Plasma ROC analysis for miR-143-3p showed poor diagnostic performance (AUC = 0.597, $p = 0.20$).

Conclusion

miR-210-3p is consistently elevated in both plasma and aqueous humor of POAG patients, supporting its potential as a minimally invasive diagnostic biomarker. miR-143-3p shows ocular-specific upregulation and may provide complementary information on local disease mechanisms. Larger multicenter studies are warranted to validate these findings and explore their clinical utility in early POAG detection.

Key points

• Primary open-angle glaucoma, being one of the leading causes of irreversible blindness, requires biomarkers for its early diagnosis, our study found that a microRNA molecule, miR-210-3p is higher in people with primary open-angle glaucoma compared to cataract patients and healthy individuals both in blood and in aqueous humor.

• miR-210-3p could distinguish glaucoma patients using a blood test highlighting its promise as a minimally invasive diagnostic marker.

• Another microRNA, miR-143-3p, was increased only in aqueous humor in eye suggesting that it may reflect local disease processes inside the eye rather than serving as a blood based diagnostic marker.

1. INTRODUCTION

MicroRNAs (miRNAs) are 18 to 22 nucleotides long, non-coding RNAs that play a central role in post-transcriptional gene regulation by acting as imperfect sequence guides and binding to target messenger RNAs (mRNAs), leading to their translational repression or degradation (1). While their primary function is to inhibit protein synthesis (2), Some studies have demonstrated that miRNAs may also enhance gene expression in certain cellular contexts (3). These molecules are essential regulators of a wide array of biological pathways, including cell proliferation, differentiation, apoptosis, metabolism, immune function, and stress responses (4). Because of their stability in body fluids and strong association with pathological states, miRNAs have emerged as promising candidates for use as diagnostic and prognostic biomarkers in complex diseases (5). Like various tissues, microRNAs are critical regulators of gene expression in ocular tissues and dysregulation of miRNAs is involved in a wide range of ocular diseases, including Glaucoma(6).

Primary Open Angle Glaucoma (POAG) is a progressive, chronic optical neuropathy marked by degenerative changes of RGCs axons, leading to irreversible vision loss (7). It is the most widespread type of glaucoma, responsible for around 70% of all glaucoma cases globally(8). The global population of individuals suffering from glaucoma is about 76 million, and this number is expected to soar to 111.8 million by 2040 due to the ageing population (9). Currently, approximately 3% of individuals aged 40–80 years globally are affected by POAG. The prevalence of glaucoma in the South Asian population is 2.1%, while that of POAG is 1.6% (10) (11). POAG develops gradually, with a slow rise in intraocular pressure (IOP), which is one of the primary risk factors for optic nerve damage (12, 13). In POAG, along with several pathological changes, there is a slow but progressive blockage of the trabecular meshwork over the years, reducing outflow and causing an increase in IOP (13), which damages the posterior structures of the eye, causing perforations in the lamina cribrosa and tissues surrounding the optic nerve head (14).

Visual field testing, optical coherence tomography (OCT), and intraocular pressure (IOP) measurement are widely used clinical approaches for glaucoma diagnosis(15). However, these methods typically identify the disease only after substantial structural and functional damage to the optic nerve has occurred, thereby limiting the potential for early intervention and increasing the risk of irreversible vision

loss(16). In ophthalmology, molecular biomarkers have attracted considerable interest because of their potential to enable earlier detection of glaucoma and provide insights into disease progression (17). Reported biomarkers include oxidative stress indicators, inflammatory cytokines, and genetic variations associated with IOP regulation and optic nerve vulnerability (18, 19). More recently, microRNAs have emerged as promising biomarker candidates for glaucoma diagnosis due to their regulatory roles in ocular pathophysiology (20).

Building on this, the present study was designed to examine the expression of POAG-related microRNAs in Pakistani patients, with the aim of identifying population-specific expression patterns and evaluating their diagnostic potential.

2. METHODOLOGY

2.1 Study Design

The study has been approved by the Research Ethics Committee (REC) of Pak- Austria Fachhochschule Institute of Applied Sciences and Technology (Approval No. PAF-IAST/2024/21), as well as by the Ethical Board of Hayatabad Medical Complex (HMC) Peshawar (Approval No. 2244/HEC/B&PSC/2024) and the study was performed in accordance with the ethical standards as laid down in the Declaration of Helsinki. Informed written consent was obtained from all patients, and cataract patients were used as controls to obtain their aqueous humor, as well as age and gender matched healthy controls for obtaining the blood. A schematic overview of the study design and experimental workflow is shown in Fig. 1.

All the patients underwent ocular examinations. The intraocular pressure (IOP) was recorded using a Goldman applanation tonometer, and four mirror indentation gonioscopy was performed for assessment of anterior chamber angles. Both blood and aqueous humor samples were obtained from POAG patients undergoing trabeculectomy. Similarly, cataract patients undergoing phacoemulsification were selected as controls for aqueous humor (AH) samples, with both blood and AH collected from them. Age and gender matched individuals without any known ocular conditions served as the healthy control group. However, only blood samples were obtained from healthy controls, as collecting AH from normal individuals was not feasible. The demographic and clinical characteristics of participants are summarized in Table 1.

Table 1

Demographic and Clinical Characteristics of Study Participants Summary of demographic and clinical characteristics of the study participants in the POAG, cataract, and control groups, including ag e range, mean age, sex distribution, intraocular pressure, and comorbidities.

Group	Number of Samples	Age Range (Years)	Mean Age (Years)	Sex Distribution (M/F)	Intraocular Pressure	Comorbidities
POAG Patients	30	25–60	48.0	15M/15F	> 22 in at least one eye	Hypertension; Diabetes
Cataract Patients	30	30–70	55.0	20M/10F	< 22 in both eyes	Hypertension; Diabetes
Normal Controls	30	25–60	50	18M/12F	--	None

The exclusion criteria for patients included diagnosis of any other type of glaucoma (e.g PACG and PEXG), previous eye trauma, or eye surgery. Moreover, patients affected with POAG but prescribed with topical eye drops rather than trabeculectomy were also excluded from the study.

2.2 Sample Collection and Processing

Peripheral blood samples were collected from eligible participants who were scheduled to undergo either cataract or trabeculectomy surgery. Blood was drawn into EDTA tubes and immediately processed. Plasma was separated by centrifugation at 2000 × g for 10 min and stored at − 80°C until microRNA isolation.

Aqueous humor samples were obtained under sterile conditions in the operating room. During trabeculectomy, a 30-gauge needle was used to collect aqueous humor at the time of paracentesis. In patients undergoing phacoemulsification for cataract surgery, aqueous humor was collected just before the main incision was made, taking care not to contact the iris or lens(21). Samples were collected in sterile microtubes and stored at − 80°C until further processing for microRNA isolation.

2.3 MiRNA Selection, Extraction, and quantitative real-time polymerase chain reaction (qRT-PCR)

For this study, two microRNAs, miR-210-3p and miR-143-3p, were selected for this study based on their distinct expression patterns in POAG patients, established biological relevance, and strong statistical significance reported in previous studies (22, 23). Total microRNA was isolated from both aqueous humor (AH) and plasma samples using the PureLink™ miRNA Isolation Kit (Thermo Fisher Scientific, USA), following the manufacturer's instructions. As an exogenous control, 3.5 µL of synthetic *Caenorhabditis elegans* miR-39 (cel-miR-39) was added to each plasma and AH sample before extraction (21, 23).

cDNA synthesis was performed using the TaqMan™ MicroRNA Reverse Transcription Kit (Applied Biosystems, Cat. #4366596) according to the manufacturer's protocol. Quantitative PCR (qPCR) was conducted with TaqMan™ microRNA assays (Applied Biosystems) specific for miR-210-3p (Assay ID: 000512), miR-143-3p (Assay ID: 002249), and the endogenous reference control miR-16-5p (Assay ID: 000391). The synthesized cDNA was diluted 1:10, and all reactions were run in triplicate on a QuantStudio™ 7 Flex Real-Time PCR System (Applied Biosystems). Thermal cycling conditions were: 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 60 seconds. Relative expression levels were calculated using the $2^{\wedge -\Delta\Delta Ct}$ method. No-template controls (NTC) and reverse transcription minus (RT-) controls were included in each run to exclude contamination and genomic DNA amplification. Ct values > 40 were considered undetectable and excluded from analysis.

2.4 Statistical Analysis

All statistical analyses were performed using MedCalc v22.0 (MedCalc Software Ltd) and GraphPad Prism v9.0 (GraphPad Software, San Diego, CA, USA). Normality of Ct and fold change ($2^{\wedge -\Delta\Delta Ct}$) distributions was evaluated using the Shapiro-Wilk test. Both variables showed deviation from normality ($p < 0.05$); therefore, non-parametric tests were applied.

Differences between two groups were assessed using the Mann-Whitney U test, while comparisons among three groups were performed using the Kruskal-Wallis test with Conover's post-hoc correction for multiple pairwise comparisons. Data are presented as median and interquartile range (IQR). For visualization, relative expression was shown as fold change, but all statistical analyses were performed on ΔCt values.

Diagnostic performance was evaluated using receiver operating characteristic (ROC) curve analysis. The area under the curve (AUC) with 95% confidence intervals (95% CI) was calculated. The Youden index was used to determine the optimal cut-off, sensitivity, and specificity. p-value < 0.05 was considered statistically significant.

3. RESULTS

3.1 miR-210-3p expression in plasma and aqueous humor

In plasma, miR-210-3p Ct values differed significantly between groups (Kruskal-Wallis's test, $\chi^2 = 78.10$, df = 2, $p < 0.000001$). Median Ct values were lowest in POAG patients (29.48; IQR: 28.95–30.12) compared with cataract (32.02; IQR: 31.75–32.29) and normal controls (34.99; IQR: 34.21–35.96), indicating higher expression in POAG. Post-hoc Conover analysis confirmed that POAG Ct values differed significantly from both cataract and normal groups ($p < 0.05$).

When analyzed as fold change, as shown in Fig. 2, plasma miR-210-3p was markedly elevated in POAG patients (median 5.18; IQR: 3.65–11.31) compared with cataract (median 1.12; IQR: 0.76–1.84) and normal controls (median 1.02; IQR: 0.43–2.59) (Kruskal-Wallis $\chi^2 = 43.03$, $p < 0.0001$).

In aqueous humor (AH), Ct values of miR-210-3p showed a trend towards lower values in POAG (median 33.99; IQR: 33.36–34.75) compared with cataract controls (median 34.31; IQR: 33.85–34.81), but the difference was not statistically significant (Mann-Whitney U = 372, p = 0.25).

Fold change analysis, depicted in Fig. 3, however, demonstrated significantly higher expression in POAG (median 2.81; IQR: 1.71–4.27) compared with cataract (median 1.12; IQR: 0.47–1.91), Mann–Whitney U = 203, Z = -3.65, p = 0.0003.

3.2 miR-143-3p expression in plasma and aqueous humor

In plasma, Ct values of miR-143-3p did not differ significantly among POAG, cataract, and normal groups (Kruskal-Wallis $\chi^2 = 2.39$, p = 0.30). Median Ct values were 30.26 (IQR: 29.92–30.80) in POAG, 30.48 (IQR: 29.98–31.07) in normals, and 30.64 (IQR: 30.16–31.42) in cataract. Fold change analysis, depicted in Fig. 4, however, showed significant differences (Kruskal–Wallis $\chi^2 = 11.54$, p = 0.003). POAG patients (median 1.31; IQR: 0.75–1.70) exhibited higher expression compared with normal controls (median 0.60; IQR: 0.12–1.08, $p < 0.05$) but not compared with cataract patients (median 0.81; IQR: 0.41–1.74).

In aqueous humor, miR-143-3p Ct values were significantly lower in POAG patients (median 27.41; IQR: 26.98–27.78) than in cataract controls (median 30.18; IQR: 29.83–30.48), Mann–Whitney U = 23, Z = −6.31, $p < 0.0001$. Consistently, fold change analysis confirmed higher expression in POAG (median 8.24; IQR: 7.44–8.85) compared with cataract (median 6.22; IQR: 4.35–7.14), Mann–Whitney U = 108, Z = −5.06, $p < 0.0001$, which is depicted in Fig. 5.

Fold change values of miR-210-3p and miR-143-3p in plasma and aqueous humor are summarized in Table 2.

Table 2

Fold change values of miR-210-3p and miR-143-3p in plasma and aqueous humor. Relative expression was calculated using the $2^{\wedge-\Delta\Delta Ct}$ method. Median (IQR) fold change values are reported. Kruskal-Wallis test with post-hoc Conover analysis or Mann-Whitney U test was applied. Fold change values are provided for interpretability, while statistics were performed on ΔCt values.

miRNA	Sample type	Group	Median fold change (IQR)	Statistical test	p-value
miR-210-3p	Plasma	POAG	5.18 (3.651–1.31)	Kruskal-Wallis χ^2 = 43.03	< 0.000001
		Cataract	1.12 (0.76–1.84)		
		Normal control	1.02 (0.43–2.59)		
miR-210-3p	AH	POAG	2.81 (1.71–4.27)	Mann-Whitney U = 203, Z = -3.65	0.0003
		Cataract	1.12 (0.47–1.91)		
miR-143-3p	Plasma	POAG	1.31 (0.75–1.70)	Kruskal-Wallis χ^2 = 11.54	0.003
		Cataract	0.81 (0.41–1.74)		
		Normal control	0.60 (0.12–1.08)		
miR-143-3p	AH	POAG	8.24 (7.44–8.85)	Mann-Whitney U = 108, Z = -5.06	< 0.0001
		Cataract	6.22 (4.35–7.14)		

3.3 ROC curve analysis

Plasma miR-210-3p showed strong diagnostic accuracy for discriminating POAG patients from controls (Fig. 6), with an AUC of 0.862 (95% CI: 0.751–0.936, p < 0.0001). At the optimal cut-off (> 3.47-fold change), sensitivity was 86.2% and specificity was 87.9%. By contrast, plasma miR-143-3p showed poor discriminatory ability (AUC = 0.597, 95% CI: 0.463–0.722, p = 0.20) (Fig. 7). The diagnostic performance of plasma miR-210-3p and miR-143-3p is shown in Table 3.

Table 3

ROC curve analysis for plasma miRNAs as potential biomarkers of POAG. Diagnostic performance of plasma miR-210-3p and miR-143-3p in distinguishing POAG patients from controls. AUC, 95% CI, optimal cut-off, sensitivity, and specificity are reported.

miRNA	AUC	95% CI	p-value	Cut-off (fold change)	Sensitivity	Specificity
miR-210-3p	0.862	0.751–0.936	< 0.0001	> 3.47	86.2%	87.9%
miR-143-3p	0.597	0.463–0.722	0.20 (ns)	–	–	–

4. DISCUSSION

In this study, we investigated the expression of two candidate microRNAs, miR-210-3p and miR-143-3p, in plasma and aqueous humor (AH) samples from patients with primary open-angle glaucoma (POAG), cataract patients, and healthy controls. Our results revealed distinct expression patterns: miR-210-3p was significantly elevated in both plasma and AH of POAG patients, while miR-143-3p showed significant upregulation only in AH but not in plasma. These findings suggest that miR-210-3p may serve as a systemic biomarker for POAG, whereas miR-143-3p reflects localized disease activity within ocular tissues, particularly the trabecular meshwork (TM).

Our data aligns with the growing body of evidence that highlights the importance of microRNAs in POAG pathogenesis and diagnosis. A recent systematic review and meta-analysis by Rezaei et al. (24) evaluated the diagnostic potential of circulating miRNAs and identified miR-210-3p as one of the most frequently validated biomarkers across different populations. The reported sensitivity and specificity of miR-210 in POAG were within ranges like those observed in our study, where plasma miR-210-3p yielded an area under the curve (AUC) of 0.862 with 86.2% sensitivity and 87.9% specificity, thus highlighting its potential as a robust non-invasive biomarker.

Previous experimental studies have demonstrated that miR-210 is upregulated under hypoxic conditions in retinal and neural tissues. Wang et al. reported that miR-210 apoptosis of neural progenitor cells(25), while Seong et al. (2025) found that persistent upregulation of miR-210 was associated with RGC loss in hypoxia-induced retinal injury models (26). Also, elevation of miRNA-210-3p increases the apoptosis of neuronal cells through the activation of the HIF-1α-VEGF signaling pathway (27). Following this, inhibition of mir-210-3p in mouse models has demonstrated reversal of elevated intraocular pressure and decreased ECM deposition in cultured human TM cells (28). A recent study has observed that hypoxic injury in RGCs induces significant upregulation of miRNA-210-3p (29). Also, another recent work performed by integrating molecular profiling with advanced machine learning approaches has also identified that miR-210-3p expression was associated with apoptosis occurrence in trabecular meshwork cells(30).

Our observation of elevated miR-210-3p in both plasma and AH of POAG patients is consistent with these mechanistic findings, reinforcing the notion that hypoxia-driven pathways contribute significantly to POAG pathology(31).

4.1 Mechanistic implications of miR-210-3p dysregulation

miR-210-3p is widely regarded as the “master hypoxa-mir” due to its robust induction under hypoxic conditions and its broad regulatory effects on mitochondrial metabolism, angiogenesis, and cell survival. One of its best characterized targets is ISCU, a critical component of the iron–sulfur cluster biogenesis pathway. Inhibition of ISCU by miR-210-3p results in mitochondrial dysfunction, impaired oxidative phosphorylation, and enhanced generation of reactive oxygen species (ROS) (31). Elevated expression of

miR-210-3p in primary pancreatic ductal adenocarcinoma tissues promotes local invasiveness by inducing mitochondrial dysfunction through suppression of ISCU expression(32).

Beyond mitochondrial regulation, miR-210-3p also promotes endothelial cell proliferation and angiogenesis by targeting inhibitors of VEGF signaling (33). Vascular dysregulation has been increasingly recognized as a contributor to glaucoma pathophysiology, with impaired ocular blood flow and abnormal autoregulation linked to optic nerve damage (34). Our findings, elevated miR-210-3p in both plasma and AH supports its dual role in driving mitochondrial dysfunction and vascular abnormalities in glaucoma. Importantly, its detectability in plasma makes it a clinically attractive biomarker for minimally invasive diagnosis and monitoring. These processes are highly relevant to POAG, as accumulating evidence implicates mitochondrial damage and oxidative stress in RGC degeneration (Fig. 8).

4.2 Mechanistic implications of miR-143-3p dysregulation

In contrast, miR-143-3p has been less consistently reported in systemic circulation. Our study revealed its significant upregulation only in AH samples of POAG patients, suggesting a predominantly local role in ocular tissues. This result is in partial agreement with prior studies. Li et al. (35) demonstrated that miR-143-3p is upregulated in TM cells and contributes to aqueous humor outflow resistance by modulating actin cytoskeletal organization. Similarly, Cordes et al. reported that miR-143 influences TGF-β induced extracellular matrix (ECM) remodeling in TM cells, further linking it to POAG pathogenesis (36). RNA sequencing of aqueous humor samples from normal-tension glaucoma patients identified miR-143-3p as significantly upregulated compared with age-matched controls, along with several other miRNAs. In TM cells, miR-143 has been shown to interact with TGF-β2 signaling to promote ECM deposition and cytoskeletal rearrangement. Through activation of the RhoA/ROCK pathway, miR-143 enhances phosphorylation of myosin light chain, leading to increased TM stiffness and reduced aqueous humor outflow (36). These processes elevate intraocular pressure (IOP), a key driver of glaucomatous optic neuropathy (Fig. 9). As we observed that miR-143-3p upregulation was confined to AH not in plasma, this discrepancy between our plasma verses AH results underscores the complexity of POAG pathophysiology, where both systemic factors (e.g., hypoxia, vascular dysregulation) and local mechanisms (e.g., TM remodeling) contribute to disease progression. Also, the absence of mir-143-3p in plasma highlights the limitations of relying solely on single or a small number of microRNAs as circulating biomarkers for glaucoma diagnosis.

5. CLINICAL IMPLICATIONS

The dual expression pattern of miR-210-3p and miR-143-3p suggests complementary diagnostic roles. Plasma miR-210-3p, with high diagnostic accuracy, could be developed into a minimally invasive blood-based biomarker for glaucoma early detection. AH miR-143-3p, although less practical for routine clinical use, could provide valuable insights into local TM dysfunction, particularly in advanced disease or during surgical interventions. A combined biomarker panel incorporating both systemic and ocular miRNAs

may ultimately enhance diagnostic precision, stratify patients by disease stage, and guide personalized treatment strategies.

From a broader perspective, our findings support the emerging paradigm of liquid biopsy in ophthalmology. Just as circulating tumor DNA has transformed cancer diagnostics, circulating miRNAs may revolutionize glaucoma management by enabling earlier detection and real-time monitoring of disease activity. However, further validation in large, ethnically diverse cohorts is necessary before clinical implementation.

6. STUDY LIMITATIONS

Despite these promising findings, several limitations must be acknowledged. First, our sample size was modest (30 patients per group undergoing ocular surgery), which may limit the generalizability of results. Second, AH samples were only available from patients undergoing ocular surgery, which may introduce selection bias. Third, systemic comorbidities such as diabetes and hypertension were present in some participants and may have influenced circulating miRNA profiles. Fourth, only two candidate miRNAs were analyzed in this study. While both were selected based on strong biological rationale, additional miRNAs are likely to be dysregulated in POAG and should be explored in future work. Finally functional validation of these miRNAs in cellular and animal models is necessary to confirm their mechanistic contributions to glaucoma pathogenesis.

7. FUTURE DIRECTIONS

Future studies should aim to address these limitations by recruiting larger, multicenter cohorts that encompass diverse ethnic backgrounds. Longitudinal studies are also needed to determine whether changes in circulating miRNAs precede clinical diagnosis and can predict disease progression. High-throughput transcriptomic approaches such as RNA sequencing may uncover additional population-specific miRNA signatures. Moreover, functional studies employing in vitro TM and RGC models, as well as in vivo glaucoma models, will be essential to elucidate the precise molecular mechanisms of miR-210-3p and miR-143-3p dysregulation. From a translational perspective, integrating miRNA biomarkers with imaging modalities (OCT, visual field testing) and IOP measurements may yield multimodal diagnostic algorithms that enhance early detection and personalized management of POAG.

8. CONCLUSION

This study demonstrates that miR-210-3p and miR-143-3p show distinct expression profiles in primary open-angle glaucoma (POAG). miR-210-3p was significantly elevated in both plasma and aqueous humor, linking hypoxia-driven mitochondrial dysfunction with systemic disease signatures and supporting its role as a minimally invasive circulating biomarker. In contrast, miR-143-3p upregulation was restricted to aqueous humor, reflecting localized trabecular meshwork remodeling and intraocular pressure regulation.

Together, these findings highlight a dual biomarker model: miR-210-3p as a promising plasma-based marker for POAG detection and monitoring, and miR-143-3p as a complementary ocular-specific marker of local disease activity. While these results provide important mechanistic and translational insights, validation in larger and more diverse cohorts is necessary before clinical application.

Declarations

Conflicts of interest

The authors declare no conflict of interest.

Funding

This study was supported by the Higher Education Commission's National Research Program for Universities (HEC-NRPU) under project grant No. 20-17501, awarded to Dr. Humaira Ayub, hosted at the Pak-Austria Fachhochschule Institute of Applied Sciences and Technology, Haripur.

Author Contribution

Eiman Meer (E.M) performed sample collection, wet-lab experiments and statistical analysis; prepared figures; and wrote the original manuscript. Yousaf Jamal Mahsood (Y.J.M) was involved in patient recruitment, clinical diagnosis, sample provision, and review of relevant methodology. Fazli Wahid (F.W) reviewed and edited the manuscript. Muhammad Ilyas (M.I) reviewed the project methodology. Sanna Khan (S.K) assisted in conceptualization and wet-lab experiements. Bakht Daniyal Khan (B.D.K) and Hussain Hussain (H.H) assisted with patient sample collection. Humaira Ayub (H.A) contributed to study conceptualization, funding acquisition, methodological review, supervision, and manuscript review. All authors haveread and approved the final manuscript.

Acknowledgement

We are highly obliged to Dr Jibril Hirbo, Research Assistant Professor of Medicine, Division of Genetic Medicine and Clinical Pharmacology for reviewing the manuscript and his valuable comments which helped us to improve the manuscript. We are highly grateful to all individuals who participated willingly in this study and provided samples to make this research possible.

Data Availability

The data used in study is newly generated qPCR data, and it is availible here: https://docs.google.com/spreadsheets/d/1hnJWZUhV91-UcqBv9uFVji-262SdBgUi/edit?usp=sharing&ouid=112576586745327823357&rtpof=true&sd=true

References

1. He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. Nature reviews genetics. 2004;5(7):522–31.
2. Ambros V, Lee RC. Identification of microRNAs and other tiny noncoding RNAs by cDNA cloning. RNA Interference, Editing, and Modification: Methods and Protocols: Springer; 2004. p. 131–58.
3. Valinezhad Orang A, Safaralizadeh R, Kazemzadeh-Bavili M. Mechanisms of miRNA-mediated gene regulation from common downregulation to mRNA-specific upregulation. International journal of genomics. 2014;2014(1):970607.
4. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. cell. 2004;116(2):281–97.
5. Wang J, Chen J, Sen S. MicroRNA as biomarkers and diagnostics. Journal of cellular physiology. 2016;231(1):25–30.
6. Dobrzycka M, Sulewska A, Biecek P, Charkiewicz R, Karabowicz P, Charkiewicz A, et al. miRNA studies in glaucoma: a comprehensive review of current knowledge and future perspectives. International Journal of Molecular Sciences. 2023;24(19):14699.
7. Nickells RW. The cell and molecular biology of glaucoma: mechanisms of retinal ganglion cell death. Investigative ophthalmology & visual science. 2012;53(5):2476–81.
8. Quigley HA, and Aimee T. Broman. The number of people with glaucoma worldwide in 2010 and 2020. British journal of ophthalmology. 2006;90(3):262–7.
9. Zhang N, Wang J, Li Y, Jiang B. Prevalence of primary open angle glaucoma in the last 20 years: a meta-analysis and systematic review. Scientific reports. 2021;11(1):13762.
10. Shan S, Wu J, Cao J, Feng Y, Zhou J, Luo Z, et al. Global incidence and risk factors for glaucoma: A systematic review and meta-analysis of prospective studies. Journal of global health. 2024;14:04252.
11. Banik S, Ghosh A, Debi H. The Prevalence Trend of Glaucoma by Age and Sex Difference in South Asia: A Systematic Review and Meta-Analysis of Population-Based Studies. Health Science Reports. 2025;8(3):e70542.
12. Tham YC, Cheng CY. Associations between chronic systemic diseases and primary open angle glaucoma: an epidemiological perspective. Clinical & experimental ophthalmology. 2017;45(1):24–32.
13. Goel M, Picciani RG, Lee RK, Bhattacharya SK. Aqueous humor dynamics: a review. The open ophthalmology journal. 2010;4:52.
14. Vecino E, Galdós M, Bayón A, Rodríguez F, Micó C, Sharma S. Elevated intraocular pressure induces ultrastructural changes in the trabecular meshwork. Journal of Cytology & Histology. 2015;3(1).

15. Schuster AK, Erb C, Hoffmann EM, Dietlein T, Pfeiffer N. The diagnosis and treatment of glaucoma. Deutsches Ärzteblatt International. 2020;117(13):225.
16. Bua S, Supuran CT. Diagnostic markers for glaucoma: a patent and literature review (2013–2019). Expert Opinion on Therapeutic Patents. 2019;29(10):829–39.
17. Tatham AJ, Weinreb RN, Medeiros FA. Strategies for improving early detection of glaucoma: the combined structure–function index. Clinical ophthalmology. 2014:611–21.
18. Beykin G, Goldberg JL. Molecular biomarkers for glaucoma. Current ophthalmology reports. 2019;7(3):171–6.
19. Agnifili L, Pieragostino D, Mastropasqua A, Fasanella V, Brescia L, Tosi GM, et al. Molecular biomarkers in primary open-angle glaucoma: from noninvasive to invasive. Progress in brain research. 2015;221:1–32.
20. Martinez B, Peplow PV. MicroRNAs as biomarkers in glaucoma and potential therapeutic targets. Neural regeneration research. 2022;17(11):2368–75.
21. Hindle AG, Thoonen R, Jasien JV, Grange RM, Amin K, Wise J, et al. Identification of candidate miRNA biomarkers for glaucoma. Investigative ophthalmology & visual science. 2019;60(1):134–46.
22. Liu Y, Wang Y, Chen Y, Fang X, Wen T, Xiao M, et al. Discovery and validation of circulating Hsa-miR-210-3p as a potential biomarker for primary open-angle glaucoma. Investigative ophthalmology & visual science. 2019;60(8):2925–34.
23. Hubens WH, Krauskopf J, Beckers HJ, Kleinjans JC, Webers CA, Gorgels TG. Small RNA sequencing of aqueous humor and plasma in patients with primary open-angle glaucoma. Investigative ophthalmology & visual science. 2021;62(7):24–.
24. Rezaei M, Faramarzpour M, Shobeiri P, Seyedmirzaei H, Sarasyabi MS, Dabiri S. A systematic review, meta-analysis, and network analysis of diagnostic microRNAs in glaucoma. European Journal of Medical Research. 2023;28(1):137.
25. Wang F, Xiong L, Huang X, Zhao T, Wu L-y, Liu Z-h, et al. miR-210 suppresses BNIP3 to protect against the apoptosis of neural progenitor cells. Stem cell research. 2013;11(1):657–67.
26. Seong H, Cho H-k, Hwang S, Kang SS. MicroRNA Expression in Retinal Ganglion Cells after Induction of Hypoxic Injury. Journal of the Korean Ophthalmological Society. 2025;66(10):409–18.
27. Sun J-J, Zhang X-Y, Qin X-D, Zhang J, Wang M-X, Yang J-B. MiRNA-210 induces the apoptosis of neuronal cells of rats with cerebral ischemia through activating HIF-1α-VEGF pathway. European Review for Medical & Pharmacological Sciences. 2019;23(6).
28. Zhao S, Fang L, Yan C, Wei J, Song D, Xu C, et al. MicroRNA-210-3p mediates trabecular meshwork extracellular matrix accumulation and ocular hypertension–Implication for novel glaucoma therapy. Experimental Eye Research. 2023;227:109350.
29. Chan SY, Loscalzo J. MicroRNA-210: a unique and pleiotropic hypoxamir. Cell cycle. 2010;9(6):1072–83.

30. Dobrzycka M, Sulewska A, Konopinska J, Karabowicz P, Charkiewicz A, Golaszewska K, et al. Machine learning-based identification of small RNA signatures in aqueous humor as a step toward precision diagnosis of glaucoma. Annals of Medicine. 2025;57(1):2568119.
31. Chan SY, Zhang Y-Y, Hemann C, Mahoney CE, Zweier JL, Loscalzo J. MicroRNA-210 controls mitochondrial metabolism during hypoxia by repressing the iron-sulfur cluster assembly proteins ISCU1/2. Cell metabolism. 2009;10(4):273–84.
32. Otsu T HM, Fujita K, Kobayashi D, Nakagawa N, Kurimoto K, Takami H, Nakanishi K, Umeda S, Shimizu D, Hattori N. Genome-wide microRNA Analysis Identified miR-210-3p Over-expression in Pancreatic Cancer Tissues as a Predictor of their Local Invasiveness. Anticancer Research. 2024;44(11):4709–21.
33. Wu G, Ding X, Quan G, Xiong J, Li Q, Li Z, et al. Hypoxia-Induced miR-210 Promotes Endothelial Cell Permeability and Angiogenesis via Exosomes in Pancreatic Ductal Adenocarcinoma. Biochemistry Research International. 2022;2022(1):7752277.
34. Emre M, Orgül S, Gugleta K, Flammer J. Ocular blood flow alteration in glaucoma is related to systemic vascular dysregulation. British Journal of Ophthalmology. 2004;88(5):662–6.
35. Li X, Zhao F, Xin M, Li G, Luna C, Li G, et al. Regulation of intraocular pressure by microRNA cluster miR-143/145. Scientific reports. 2017;7(1):915.
36. Cordes KR, Sheehy NT, White MP, Berry EC, Morton SU, Muth AN, et al. miR-145 and miR-143 regulate smooth muscle cell fate and plasticity. Nature. 2009;460(7256):705–10.

Figures

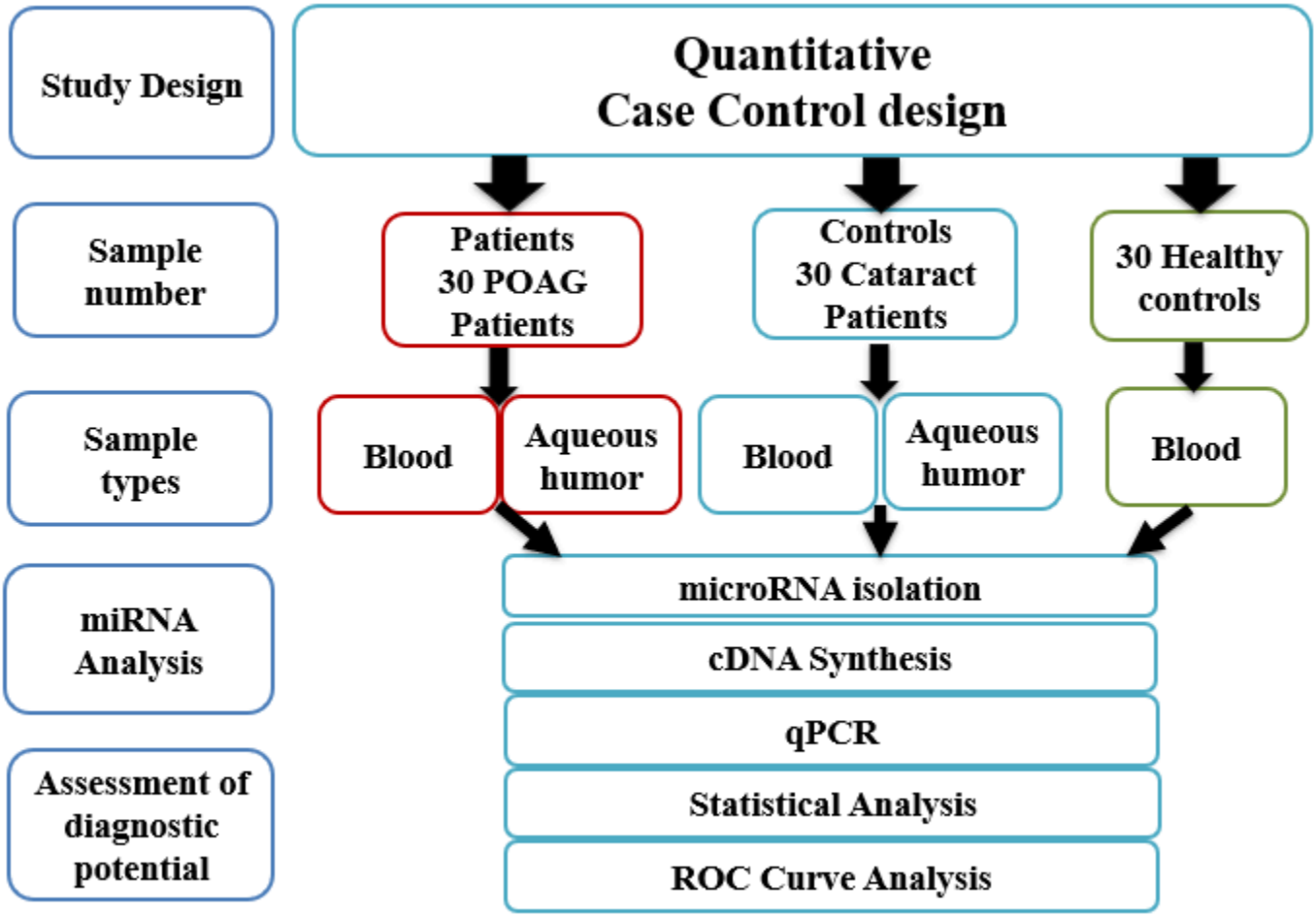


Figure 1

Study design and experimental workflow.

Schematic representation of the quantitative case–control design. Samples were collected from POAG patients (plasma and aqueous humor), cataract patients (plasma and aqueous humor), and healthy controls (plasma). Experimental workflow included microRNA isolation, cDNA synthesis, qPCR, statistical analysis, and ROC curve analysis to assess diagnostic potential.

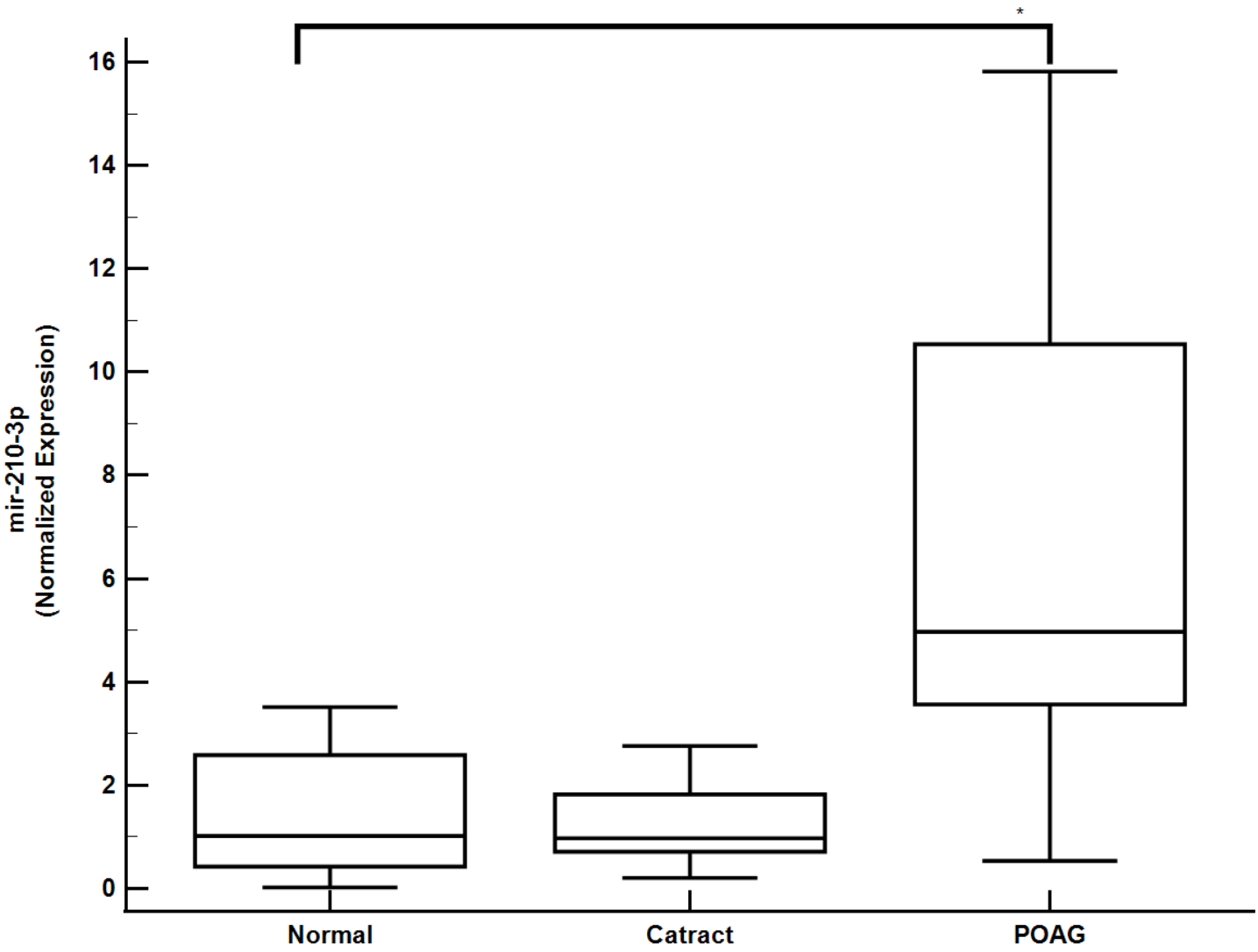


Figure 2

Relative expression of miR-210-3p in plasma across study groups.

Box-and-whisker plot showing normalized expression levels of **miR-210-3p** across study groups. A significant upregulation of miR-210-3p was observed in the POAG group compared with both cataract and normal controls ($p < 0.001$, indicated by the asterisk "*").

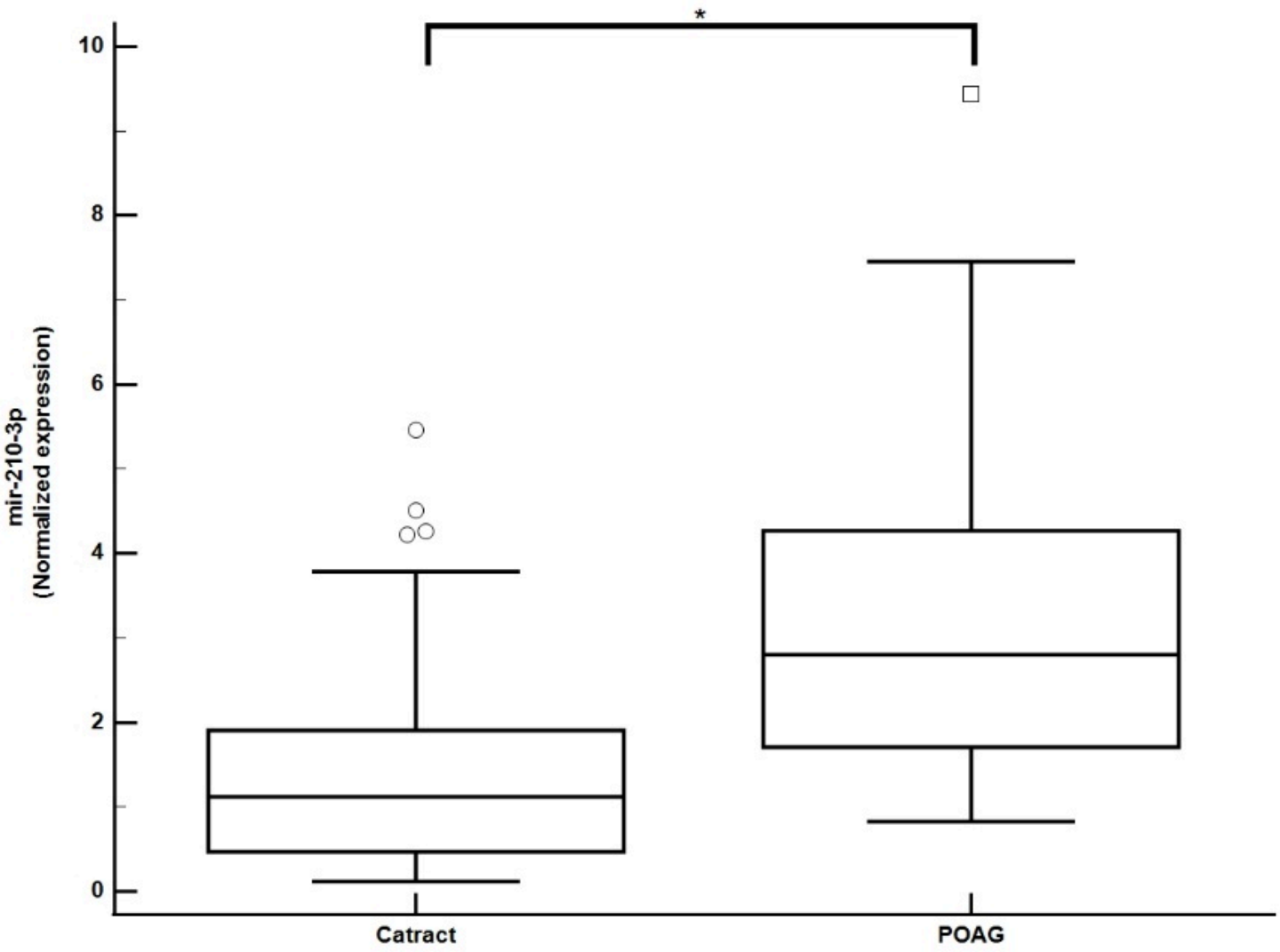


Figure 3

Relative expression of miR-210-3p in aqueous humor samples across study groups.
A significant upregulation of miR-210-3p was observed in the POAG group compared with cataract controls ($p < 0.001$ indicated by asterisk "*").

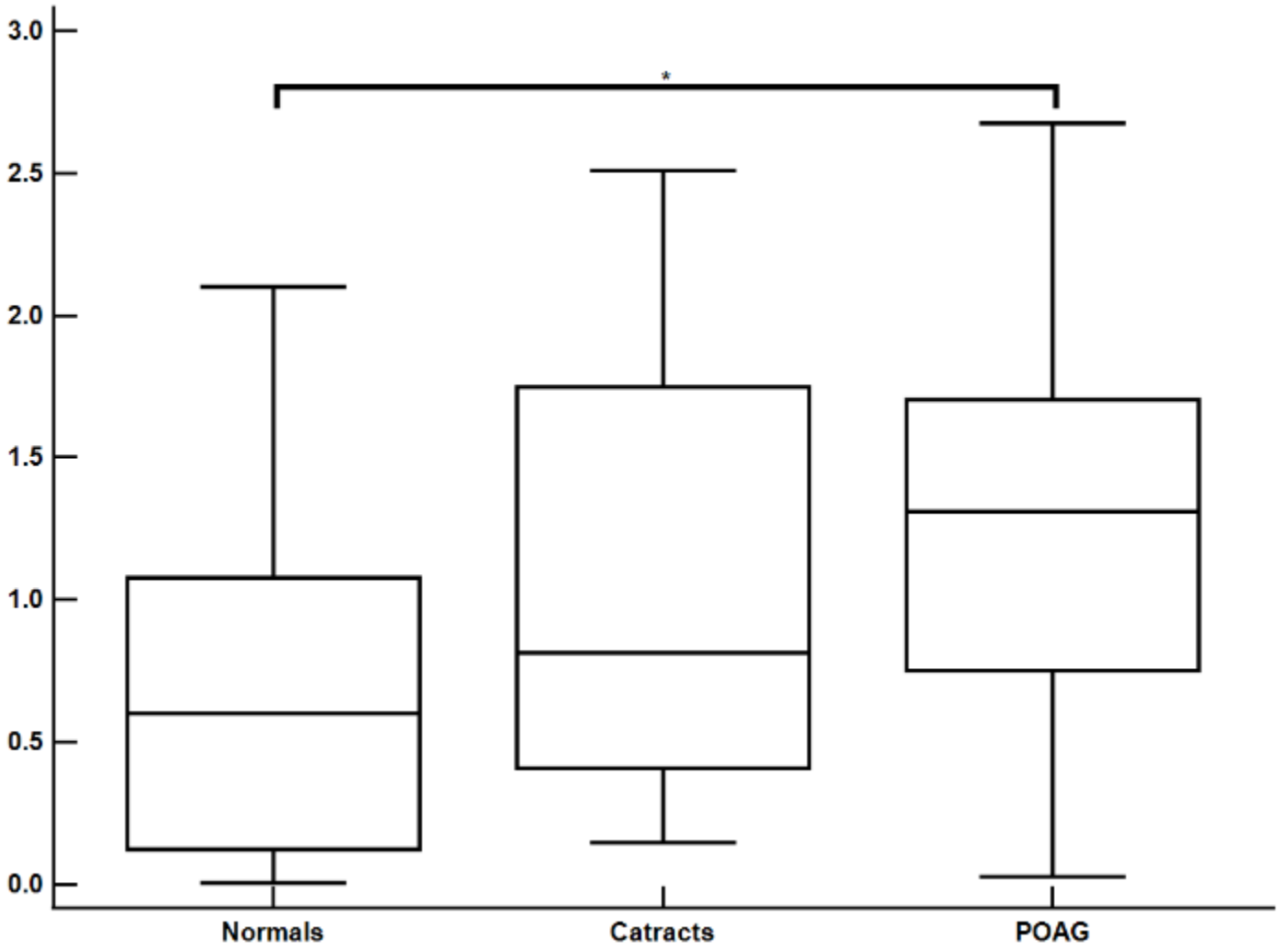


Figure 4

Relative expression of miR-143-3p in plasma across study groups.
Comparison among POAG, cataract, and normal controls but no significant differences were observed.

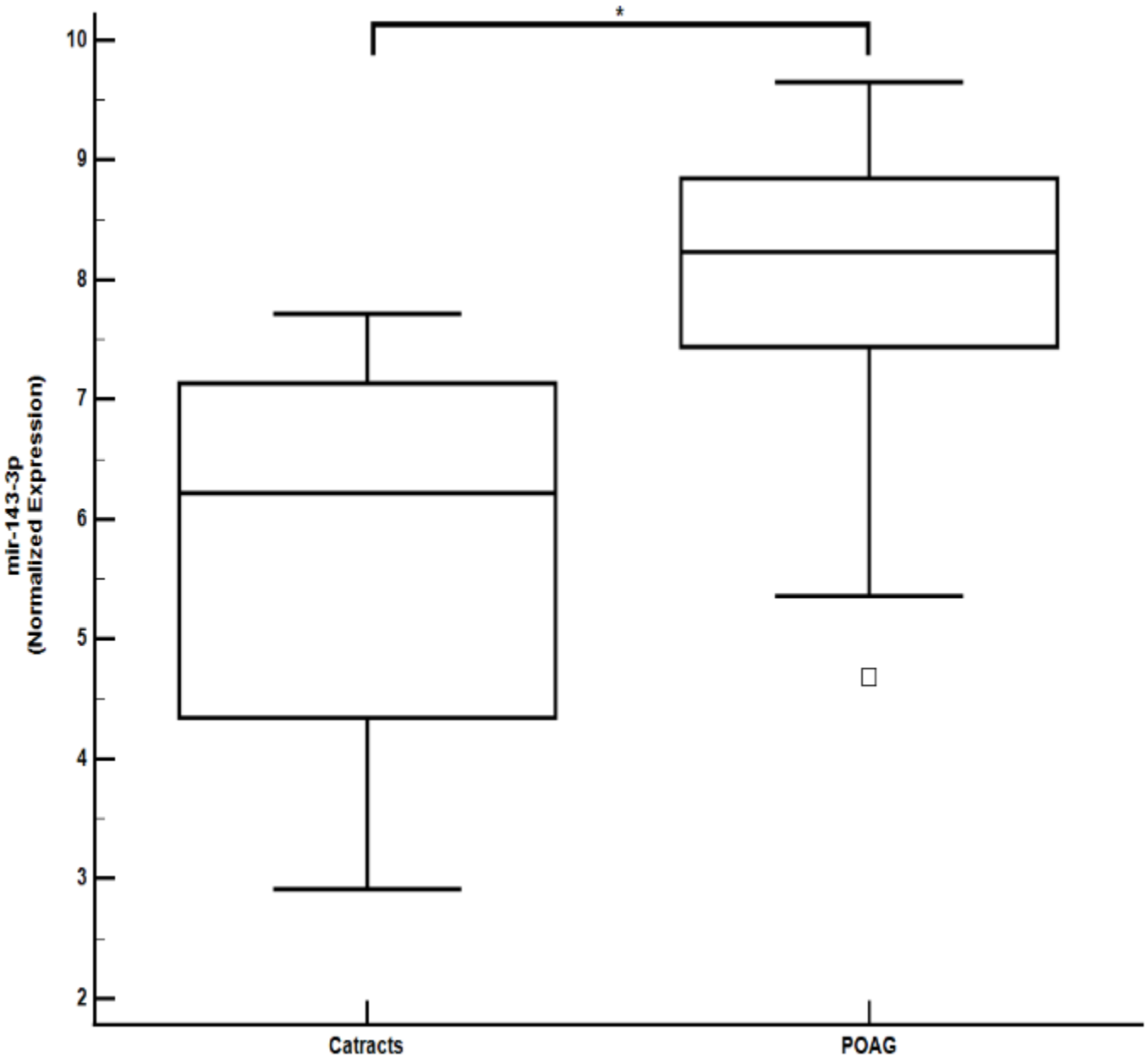


Figure 5

Relative expression of miR-143-3p in aqueous humor samples across study groups. POAG eyes demonstrated significant upregulation of miR-143-3p relative to cataract controls ($p < 0.001$ indicated by asterisk "*").

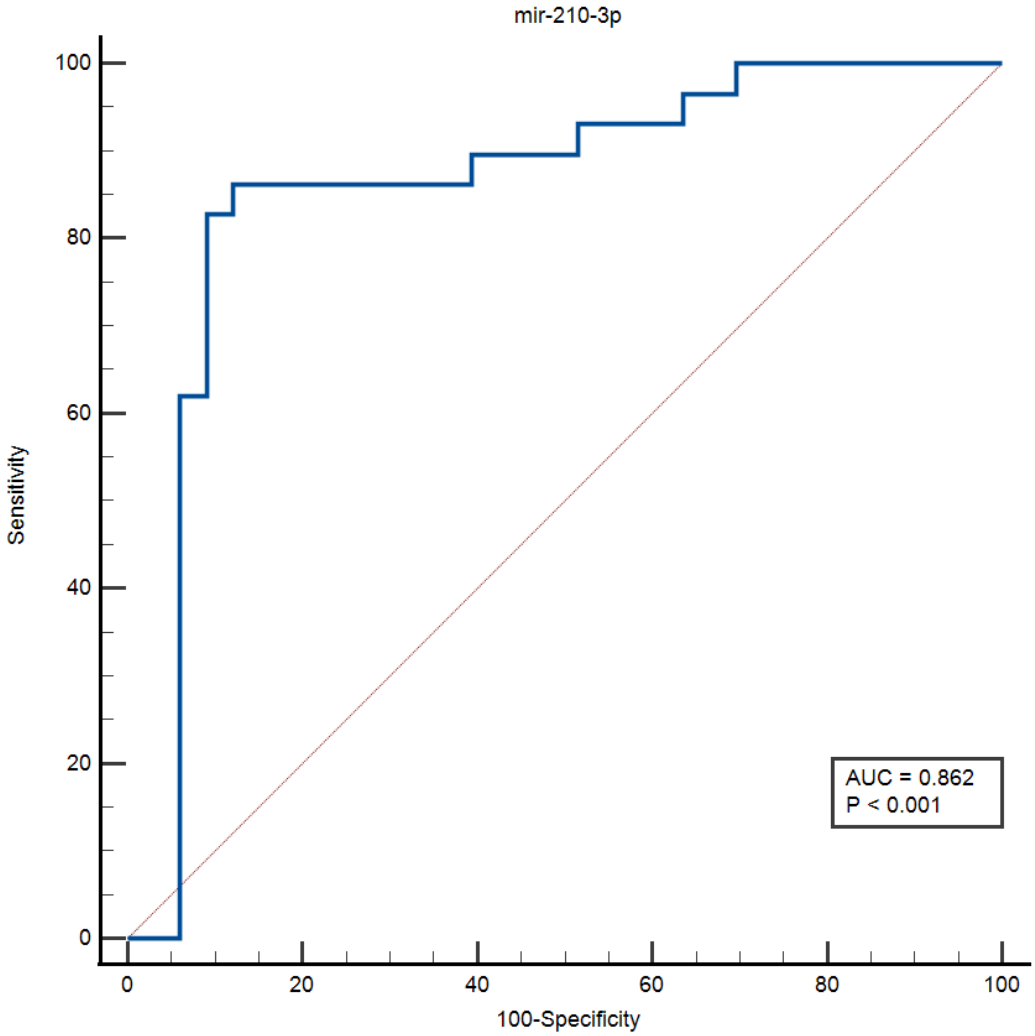


Figure 6

ROC curve for plasma miR-210-3p
Plasma miR-210-3p showed high diagnostic accuracy (AUC = 0.862, sensitivity = 86.2%, specificity = 87.9%). The area under the curve (AUC) was 0.862 (95% CI: 0.751–0.936, p < 0.0001), indicating strong discriminative ability. An optimal cut-off fold change of >3.47 was determined using Youden's index, providing a sensitivity of 86.2%, meaning that miR-210-3p correctly identified 86.2% of POAG cases, and a specificity of 87.9%, correctly classifying 87.9% of non-POAG samples (cataract and healthy controls) as negative.

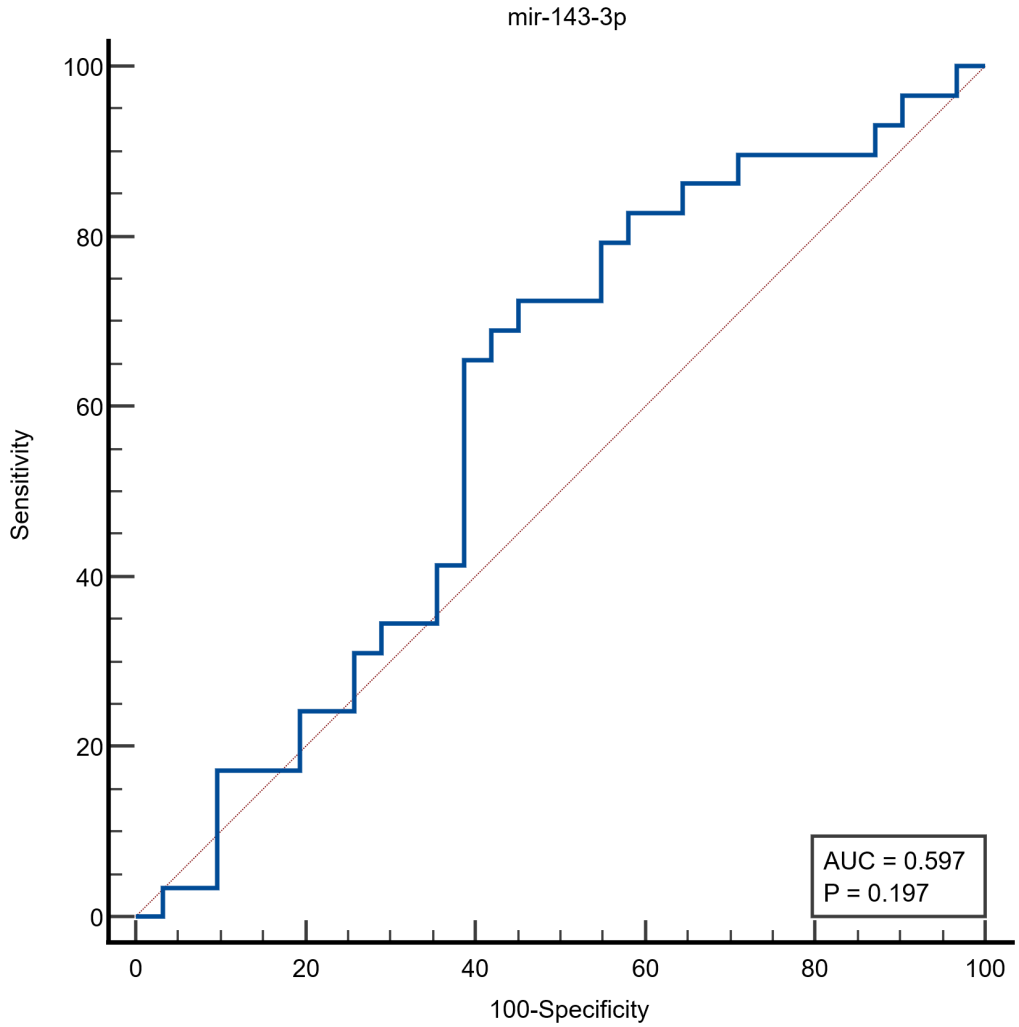


Figure 7

ROC curve for plasma miR-143-3p.

Plasma miR-143-3p demonstrated poor diagnostic accuracy (AUC = 0.597, not significant). The area under the curve (AUC) was **0.597** (95% CI: 0.463–0.722, p = 0.20), indicating poor discriminative performance and no statistical significance.

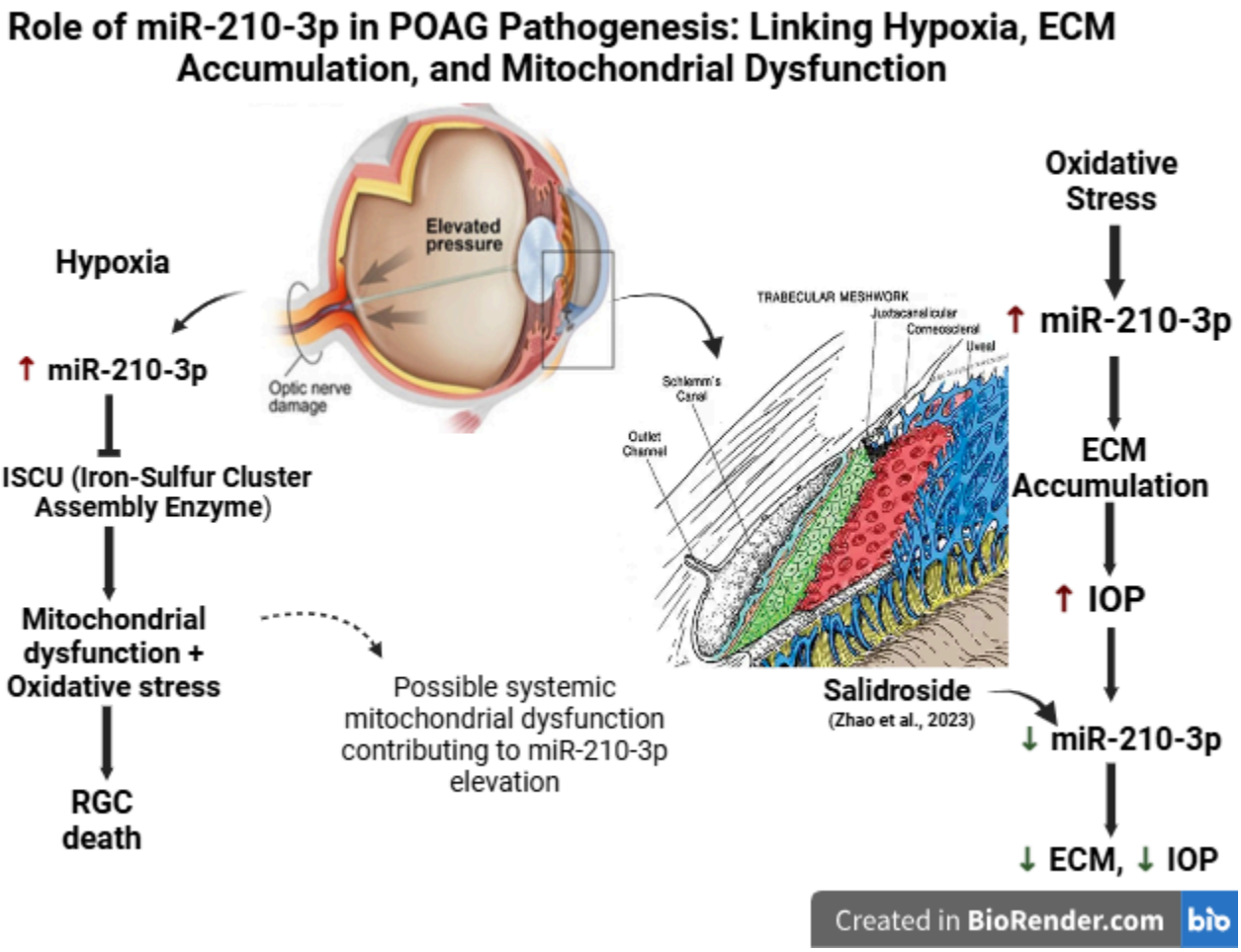


Figure 8

Proposed role of miR-210-3p in POAG pathogenesis

This figure demonstrates the versatile role of miR-210-3p in the development of primary open-angle glaucoma (POAG). Hypoxic conditions in ocular tissues elevate miR-210-3p expression, which downregulates ISCU (Iron-Sulfur Cluster Assembly Enzyme), which ultimately leads to mitochondrial dysfunction and increased oxidative stress [32]. In parallel, miR-210-3p in the trabecular meshwork promotes ECM accumulation, impairing aqueous humor outflow, and raising intraocular pressure (IOP). Natural compounds like salidroside have been shown to decrease miR-210-3p levels, thus alleviating oxidative stress, reducing ECM deposition, and lowering IOP [27].

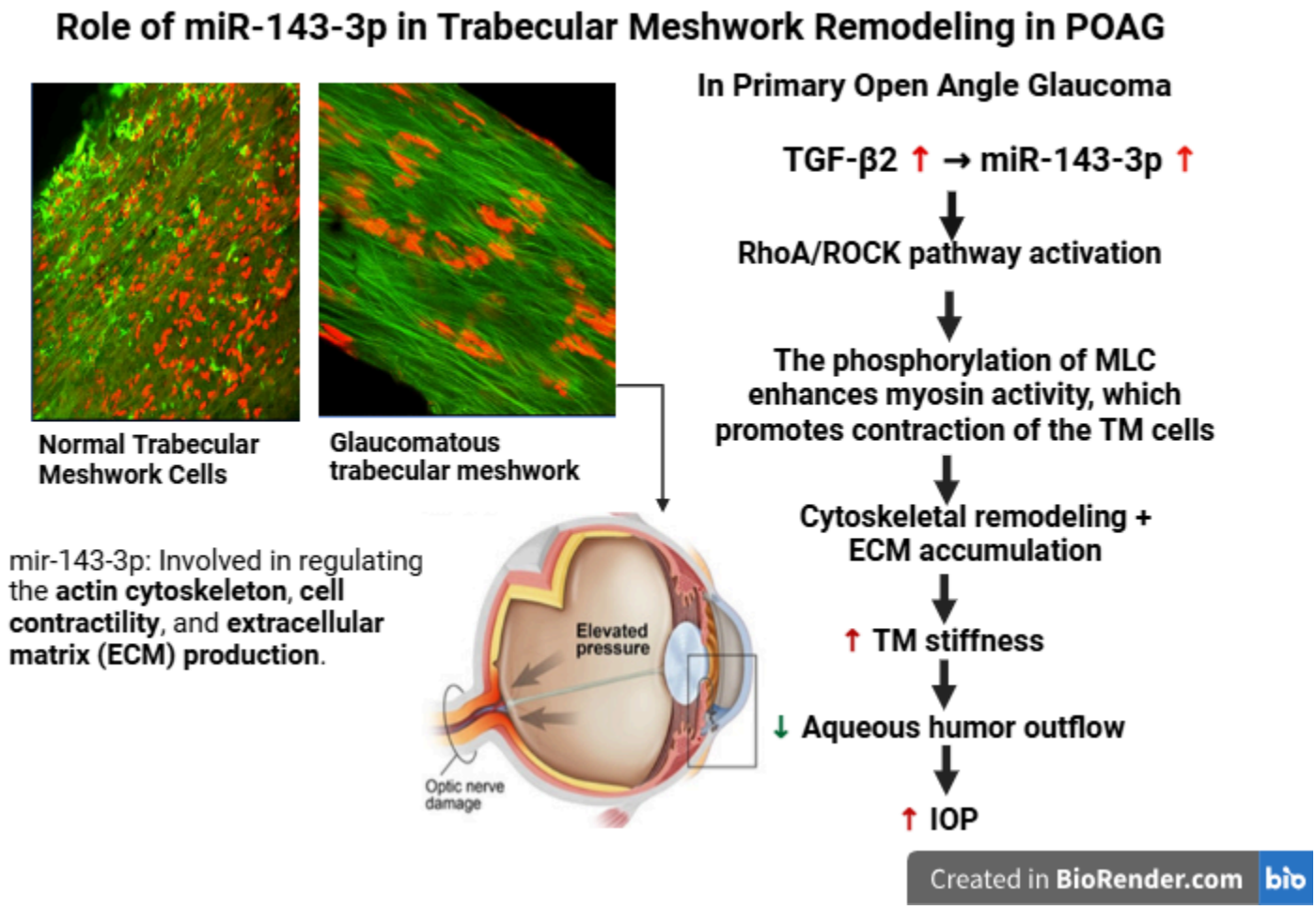


Figure 9

Proposed role of miR-143-3p in trabecular meshwork remodeling.

In glaucomatous eyes, elevated levels of TGF-β2 induce the upregulation of miR-143-3p, which subsequently activates the RhoA/ROCK signaling pathway. This pathway enhances the phosphorylation of myosin light chains (MLC), increasing myosin activity and promoting contraction of TM cells [34, 35]. These events lead to cytoskeletal remodeling, extracellular matrix (ECM) accumulation, and increased TM stiffness. The stiffening of TM impairs aqueous humor outflow, resulting in elevated intraocular pressure (IOP) and optic nerve damage [35].

Supplementary Files

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- StatisticalAnalysisforsubmission.docx