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Role of nitric oxide (NO) : Unveiling new horizons in changes of glaucoma vasculature and ocular surface among Primary Open Angle Glaucoma

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ABSTRACT

Objective: This article reviews the role of NO in POAG against the background of the vascular changes in eye and ocular surface abnormalities. NO not only plays a significant role in regulation of IOP among glaucoma patients by influencing the outflow pathways but also plays a key role in vaso regulation of optic nerve & its effects on corneal surface to modulate the disease process .The aim of this study is to alert readers to possible NO involvement in glaucoma pathophysiology and encourage them to recognize possible therapeutic relevance.

Methods: It's a cross sectional prospective observational study with 100 participants randomly divided into two distinct groups: 60 patients with POAG, and 40 healthy controls. The NO levels in plasma and aqueous humor were detected by Griess reagent assays. Optical coherence tomography angiography and Doppler ultrasound were used to measure blood flow in the eye. Clinical tests and Impression cytology studies were employed for both the groups to evaluate tear film stability and corneal integrity.Statistical analyses were performed to check if NO levels correlated with vascular changes and ocular surface parameters.

Result: NO metabolite levels were not found to be decreased significantly in POAG patients as compared to controls ($p < 0.001$). Both OCTA and Doppler ultrasound showed reduced vessel density and blood flow velocities among POAG patients, which indicated compromised optic nerve head perfusion. Tear film stability and production were highly impaired in the POAG group. Increased corneal epithelial damage was evaluated by elevated inducible nitric oxide synthase (iNOS) expression on impression cytology. Levels of NO were found to be reduced and values of both vascular inadequacy and ocular surface defects correlated significantly ($p < 0.001$).

Conclusion: It underlines the significant role NO plays in POAG and strongly connects its dysregulation to vascular and ocular surface changes that contribute to progression in disease. Therefore, pathways through which NO may be targeted suggest a potential dual therapeutic strategy improving vascular perfusion and alleviating ocular surface disease and is a promising approach that opens the road to better clinical outcomes in managing glaucoma.

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Keywords: Primary open-angle glaucoma, nitric oxide, ocular vascular changes, ocular surface disease, optic nerve perfusion, inducible nitric oxide synthase

I. INTRODUCTION

Nitric oxide (NO) is a gaseous signaling molecule that plays a crucial role in many physiological and pathological processes of the human body. No longer known simply as a vasodilator involved with cardiovascular function, NO has been implicated as a major modulator of vascular and cellular health in numerous organ systems, including ocular tissue. NO's role within the field of ophthalmology, especially regarding primary open-angle glaucoma, has developed into a new area of growing interest for researchers [1]. POAG constitutes a multifactorial condition that is chronic optic neuropathy where degeneration progresses in retinal ganglion cells that eventually leads to permanent vision loss and irreversibility unless treated. The traditional understanding has led to the positioning of high intraocular pressure (IOP) as the topmost risk factor for POAG. However, studies nowadays point out vascular dysregulation, oxidative stress, and inflammatory processes also as critical contributors to disease progression [2-3].

One of the interesting areas of research in glaucoma includes vascular changes associated with NO signaling. NO produced by endothelial cells within the eye is essential in maintaining the ocular blood flow via its vasodilatory impact on the blood vessels. Diminished or ectopic NO levels within the ocular microvasculature have been surmised as one of the causes of the distorted blood flow seen in glaucoma pathogenesis, particularly at the optic nerve head. This interruption in blood flow leads to apoptosis of retinal ganglion cells, worsening the optic nerve damage characteristic of POAG. Accordingly, knowing the vascular dynamics associated with NO dysregulation in newer studies may indicate novel therapeutic approaches to protect the optic nerve in patients suffering from glaucoma [4-5].

Furthermore, NO has significant effects on the ocular surface, influencing both tear film stability and corneal health [6].

This means that the chronic treatment of POAG patients with anti-glaucoma medications changes NO levels in ocular surfaces, potentially triggering ocular surface disease. Such a disease is marked with inflammation, tear instability, and discomfort, which might reduce patient adherence to glaucoma therapy; therefore, treatments that serve both intraocular and ocular surface health are required. In that respect, a full investigation of NO involvement in alterations at the ocular surface in patients suffering from glaucoma promotes hopes to improve patient life and treatment efficacy. The horizon of the role of NO in the pathophysiology of glaucoma is constantly expanding, and we concentrate our focus on its contribution to the regulation of ocular blood flow and to the integrity of the ocular surface in POAG. We consider that a discussion of recent advances and mechanistic insights into NO signaling pathways will throw light on new therapeutic targets as well as new treatment modalities potentially improving the outcomes in the management of glaucoma [7-8].

II. METHODS

Study Design and Participants

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It was a Prospective, Cross sectional, Observational study conducted at a tertiary care in the Department of Ophthalmology of central India over the period of 12 months. It consisted of 100 patients belonging to 2 groups divided randomly:

1. **Group 1 : POAG group:** 60 patients diagnosed with POAG on the basis of an elevated intraocular pressure, optic disc changes, and visual field loss.

2. **Group 2 : Control group:** 40 age- and gender-matched healthy persons without any history of diseases affecting NO signaling either ocularly or systemic in nature.

Institutional Ethical and Scientific approval was obtained, and written informed consent was taken from every participant before the survey.

I. CLINICAL EVALUATION

- Best corrected visual acuity (BCVA) both for distance and near were taken for both groups through Snellens chart.
- Intraocular Tension (IOP) was measured through the gold standard method of Goldmann Applanation Tonometer at a particular time of day.
- Thorough anterior segment evaluation done for both groups via slit lamp and dilated posterior segment evaluation was performed by +90 D lens.
- Perimetry was performed by Zeiss static perimeter under standard 24-2 program.
- The two variables of IOP and VF were tested between both groups in order to correlate vascular and surface examinations with functional severity of glaucoma.

II . ASSESSMENT OF ALTERNATION OF OCULAR SURFACE

Tear Film and Corneal Tests

Tests done to determine the ocular surface health included:

1. Tear Break-Up Time (TBUT): Measured tear film stability on corneal surface to determine surface integrity . A TBUT value of 10-35 sec is considered normal.
2. Schirmer's test-Measured quantity of tear produced. Fluorescein dye with slit-lamp microscopy showed epithelial damage by corneal staining.Both Schirmer I and II were performed.

III. ASSESSMENT OF OCULAR VASCULAR CHANGES

1. Measurement of Nitric Oxide Level

NO levels were assayed using blood plasma and aqueous humor samples that were simultaneously obtained by performing aqueous humor tap, for patients of both groups with POAG and controls.The stable metabolites of NO, nitrite and nitrate, were measured using a Griess reagent assay.

2. Ocular Blood Flow Analysis

The microvasculature of the optic nerve head and retina was identified using OCTA, and comparisons were made between POAG patients and controls about retinal and optic nerve

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head vessel density and perfusion indices. Blood flow velocities in the central retinal artery and ophthalmic artery were assessed by Doppler ultrasound.

IV . IMPRESSION CYTOLOGY

Conjunctival epithelial cells were isolated using impression cytology, by placing the blot paper on both superior and inferior bulbar conjunctiva and their inflammatory cytokine elaboration and NO synthase activity were probed. Inducible nitric oxide synthase activity was studied by immunohistochemical method.

Statistical Analysis

Data were analyzed using SPSS software version 27. Data are presented as means ± SD. The quantitative variables between the groups were compared using an independent t-test and Mann-Whitney U tests. Pearson's correlation coefficient was used for correlation of NO levels, ocular blood flow, and surface changes. Results with a p-value < 0.05 are considered statistically significant.

III. RESULTS

A. NITRIC OXIDE LEVEL COMPARISON

The NO metabolite levels (nitrite and nitrate) were significantly reduced in the aqueous humor and plasma samples of the POAG group compared to the controls. Mean aqueous humor nitrite levels were 5.6 ± 0.9 µM in the POAG group versus 9.2 ± 1.3 µM in the control group ($p < 0.001$). Plasma nitrate levels followed a similar trend, with lower concentrations in POAG patients (15.4 ± 3.7 µM) compared to controls (23.8 ± 4.1 µM, $p < 0.001$). *Table 1* summarizes the NO metabolite levels.

Table 1: Nitric Oxide Metabolite Levels

Sample Type	POAG Group (Mean ± SD)	Control Group (Mean ± SD)	p-value
Aqueous Humor (µM)	5.6 ± 0.9	9.2 ± 1.3	<0.001
Plasma (µM)	15.4 ± 3.7	23.8 ± 4.1	<0.001

B. OCULAR VASCULAR CHANGES COMPARISON

Optical Coherence Tomography Angiography (OCTA)

Vessel density in the superficial and deep retinal capillary plexus was found to be significantly reduced in POAG patients. The optic nerve head perfusion index was 37.6 ± 4.2% in the POAG group compared to 45.8 ± 3.9% in controls ($p < 0.001$).Figure 1 summarizes the OCTA values.

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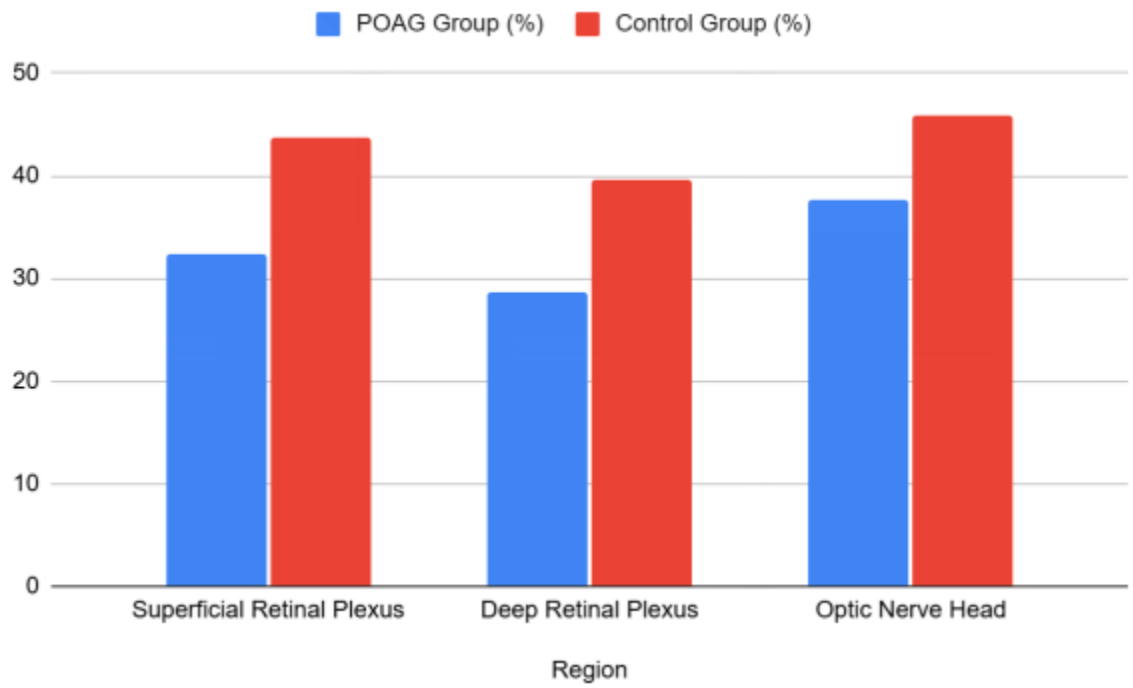

Figure 1: OCTA images comparing vessel density in POAG and control groups.

Figure 1 illustrates OCTA images showing vessel density differences, while *Table 2* presents the Doppler ultrasound measurements.

Doppler Ultrasound

Reduced blood flow velocities were observed in the central retinal artery (9.4 ± 1.2 cm/s in POAG vs. 13.8 ± 1.5 cm/s in controls, $p < 0.001$) and ophthalmic artery (24.2 ± 3.3 cm/s in POAG vs. 31.6 ± 4.2 cm/s in controls, $p < 0.001$).

Table 2: Doppler Ultrasound Findings

Vessel	POAG Group (cm/s)	Control Group (cm/s)	p-value
Central Retinal Artery	9.4 ± 1.2	13.8 ± 1.5	<0.001
Ophthalmic Artery	24.2 ± 3.3	31.6 ± 4.2	<0.001

Table 3: Ocular Surface Parameters

Parameter	POAG Group	Control Group	p-value
TBUT (seconds)	6.4 ± 1.1	11.8 ± 2.3	<0.001
Schirmer's Test (mm)	8.2 ± 2.6	14.5 ± 3.1	<0.001

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Corneal Staining (Score)	2.6 ± 0.8	0.9 ± 0.4	<0.001

C. OCULAR SURFACE CHANGES COMPARISON

Tear Film and Corneal Changes

Tear film stability and production were significantly impaired in the POAG group. The mean TBUT was 6.4 ± 1.1 seconds in POAG patients versus 11.8 ± 2.3 seconds in controls ($p < 0.001$). Schirmer's test values were also lower in the POAG group (8.2 ± 2.6 mm vs. 14.5 ± 3.1 mm, $p < 0.001$).

Fluorescein corneal staining scores indicated more severe epithelial damage in POAG patients (2.6 ± 0.8) compared to controls (0.9 ± 0.4, $p < 0.001$).

Table 3: Ocular Surface Parameters

Parameter	POAG Group	Control Group	p-value
TBUT (seconds)	6.4 ± 1.1	11.8 ± 2.3	<0.001
Schirmer's Test (mm)	8.2 ± 2.6	14.5 ± 3.1	<0.001
Corneal Staining (Score)	2.6 ± 0.8	0.9 ± 0.4	<0.001

Impression Cytology Findings

Conjunctival impression cytology showed increased expression of inducible nitric oxide synthase (iNOS) in POAG patients. These findings correlated with tear film instability and higher corneal staining scores.

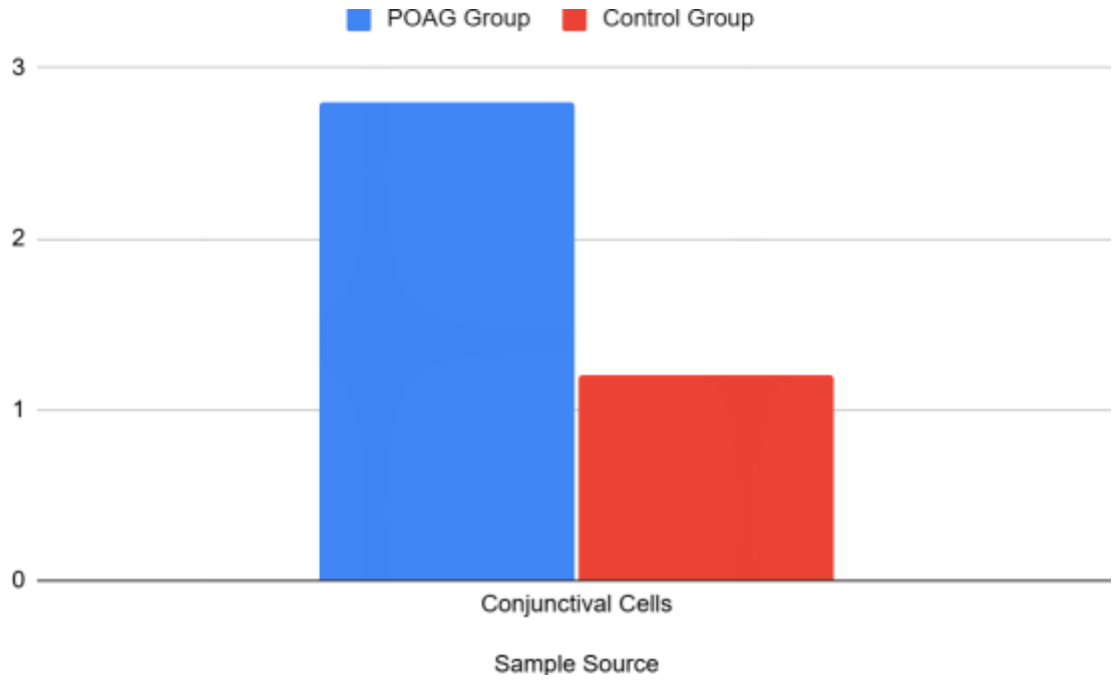

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Figure 2: Impression cytology samples showing elevated iNOS expression in POAG patients

Figure 2 shows representative cytological samples, and *Table 3* provides a comparative summary of ocular surface parameters.

Correlation Analysis

Significant correlations were observed between reduced NO levels and both vascular and ocular surface abnormalities. Lower NO metabolite levels were associated with reduced vessel density ($r = 0.65$, $p < 0.001$) and shorter TBUT ($r = 0.58$, $p < 0.001$).

IV. DISCUSSION

This study is pretty good evidence for the involvement of NO in the pathophysiology of primary open-angle glaucoma, and it draws attention to its influence on vascular changes and ocular surface health of such patients. Its findings point toward the complexity of glaucoma beyond having just elevated intraocular pressure; however, it focuses on the progress of the disease based on multifactorial contributions from vascular dysregulation and ocular surface abnormalities [9-10].

Lower levels of NO metabolites in both aqueous humor and plasma of POAG patients indicate a systemic and localized deficiency of NO signaling. NO is a well-known regulator of vascular tone; depletion may compromise microvascular function and subsequently result in inadequate perfusion of the optic nerve head. Observations that include diminished vessel density of the retinal and optic nerve head as noted by OCTA tend to strengthen this hypothesis. Additionally, the marked reduction in blood flow velocities within the central retinal and ophthalmic arteries supports the role of vascular insufficiency in glaucomatous optic neuropathy. These findings were consistent with literature; NO deficiency has been known to exacerbate both hypoxia and oxidative stress within the optic nerve head, thereby promoting the apoptotic action upon the retinal ganglion cells [11-12].

The ocular surface alterations in the POAG patients also form the basis of NO dysregulation complexity. The instability of the tear film, low tear volume, and higher damage to corneal epithelium all were found significantly higher within the POAG group. In impression cytology, there was an upregulation noted for iNOS indicative of a potential inflammatory response due to long-term antiglaucoma therapy or from underlying disease processes. These changes are clinically significant as they not only interfere with the comfort and quality of life of patients but may also compromise adherence to the glaucoma treatment regimen [13].

The reduced NO level correlates with vascular as well as ocular surface abnormalities, underlining the interdependency of these two systems in the maintenance of ocular health. Because NO has vasodilatory activity it is an important chemical molecule for preserving ocular blood flow, and further help in maintaining the integrity of the ocular surface respecting its properties as an anti-inflammatory and modulator of cellular signaling. The findings of the study, therefore, reveal dual benefits for POAG patients by favoring therapeutic strategies targeting NO pathways-both enhancing vascular perfusion to the optic nerve head and slowing ocular surface disease [14].

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Such findings are replete of several therapeutic implications. Preclinical studies using pharmacologic agents which increase NO availability or mimic its effects, such as NO-donor molecules, are promising treatments. Other treatments may include anti-inflammatory therapies to improve ocular surface health, especially the formulation of preservative-free antiglaucoma medications. The future of clinical studies should lie on the synergistic effect of these therapeutic strategies which may potentially offer a more holistic approach for glaucoma management [15].

Certain limitations are inherent in this study, and these need to be recognized. Cross-sectional design does not allow establishing causality between dysregulation of NO with progression in glaucoma. Longitudinal studies would be required for testing whether NO level restoration would arrest or reverse vascular and ocular surface changes. The sample size is relatively small and, therefore, the findings might limit to a smaller population; further studies should be undertaken with increased and diverse populations.

In brief, NO comes out as pivotal in POAG and first time links its dysregulation with vascular insufficiency and ocular surface disease. Such findings will not only enlarge the horizon of understanding glaucoma pathophysiology but also open new avenues for targeted therapeutic interventions. No wonder, NO-based therapies might offer a holistic way to target vascular and ocular surface abnormalities simultaneously that may improve the outcomes of patients suffering from glaucoma.

V. CONCLUSION

The present study highlights the dual role of NO in the pathogenesis of POAG by demonstrating its crucial role in the development of ocular vascular dysregulation as well as ocular surface abnormalities. The decrease level of NO has been shown to be related with the poor optic nerve head perfusion and microvascular insufficiency which increases the loss of retinal ganglion cells along with inflammation of the ocular surface and destabilization of tear film. These findings spur towards the requirement of therapeutic interventions of NO pathways and enhance the vascular health with relief of ocular surface diseases which will be operated on through a dual approach of improving the clinical outcome and quality of life of the glaucoma patient. Thus integrative understanding of the role of Nitric oxide(NO) accords great promise as a potential target in POAG management.

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