Metabolomics and Pharmacogenomic Evaluation of Glaucoma Therapy

Introduction

Glaucoma is characterised as optic neuropathy due to the death of retinal ganglion cells (RGC) which results in the irreversible loss of vision (Casson et al., 2012). It is considered as a multifactorial disease as several mechanisms have been postulated for the death of RGCs including, elevated IOP, cellular apoptosis (Guo et al., 2005; McKinnon, 1997), vascular insufficiency (Ster et al., 2014; Ahmad, 2016), neuroinflammation (Soto et al., 2014), autoimmune response (Shazly et al., 2011), oxidative stress, mitochondrial dysfunction (Chrysostomou et al., 2013), glutamate excitotoxicity (Salt et al., 2006), etc. Estimated global prevalence of glaucoma will be estimated to reach up to 112 million in 2040 (Tham et al., 2014). India accounts for around 12 million glaucoma cases, which is one-fifth of the global glaucoma burden (George et al., 2010). Primary Open Angle Glaucoma (POAG) and primary angle closure glaucoma (PACG) are highly prevalent and two of the most common forms of all primary adult glaucoma (PAG) (Gupta et al., 2016) (Sharts-Hopko et al., 2009). The highest prevalence of open-angle glaucoma occurs in Africans, and the Asians have highest prevalence of angle-closure glaucoma (Cook et al., 2012). However, glaucoma is still incurable because the currently available treatment modalities focus only on the symptomatic approach of controlling the elevated IOP (majority of the glaucoma patients have an IOP >21mmHg), which is one of the major risk factors for glaucoma. Therefore, exploring the detailed understanding on the pathogenic mechanism involved in glaucoma is crucial to optimize the current therapeutic options available for glaucoma and thereby introduce a newer approach towards optic neuroprotection (Vasudevan et al., 2011). Due to the multi-factorial nature of glaucomatous aetiology, precision medicine will become the prerequisite for forthcoming glaucoma therapeutics. Since the last decade, multi-omics strategies and tools for early diagnosis of glaucoma have rampantly progressed to make it more promising. It is very crucial to understand the disparities of biological pathways in case of glaucoma (Lauwen et al., 2017). High throughput and sophisticated technological advances like High resolution mass spectrometry (HRMS) & Nuclear Magnetic Resonance (NMR)-based omics profiling brings out the possibility of identifying the potential biomarkers, exploring newer therapeutic targets and understanding the mechanisms of different molecular pathways intricated in glaucoma progression (Marshall et al., 2017). Therefore, profiling and integrating the different levels of omics (metabolomics, lipidomics and proteomics) data by utilizing the network and pathway analysis may bring out the new dimensions for therapeutic outcome. Inter-individual differences are more common towards their drug response. The advent of pharmacogenomic approach enables to screen SNPs and analyse the variation in their genotype frequencies of different molecular targets, which could in turn, highly influence the pharmacodynamic response (McLaren et al., 2003). There is a lack of knowledge in understanding the multiomic variations associated with PACG. Till date, no comprehensive studies correlated

the impact of systemic alterations in omics pathways to decipher the localized changes associated with glaucoma pathology. In context with current anti-glaucoma medications, prostaglandin analogues and beta blockers remain first line of treatment for POAG. Understanding the distribution of polymorphism in their pharmacokinetic and pharmacodynamic pathways may help optimize the drug treatment based on individual response for an emerging era of personalised medicine. Only limited number of studies has been carried out to find the association of ADRB2 and OATP2A1 polymorphism with open angle glaucoma. Currently, there are no studies available in Indian population. Therefore, the primary aim of the present study was to identify both the localized and systemic alterations in multi-omic profiles associated with POAG and PACG using HRMS. The secondary aim is to understand the association of SNPs present in ADRB2 and OATP2A1 in patients with POAG.

Objectives

To achieve this aim following objectives were framed as mentioned below:

1. Primary metabolite profiling in aqueous humor of glaucomatous patients using NMR
2. Untargeted metabolomics in glaucomatous patients using high resolution mass spectrometry
3. Profiling proteomic variation in glaucomatous patients using High Resolution Mass spectroscopy
4. Profiling lipidomic variation in glaucomatous patients using High Resolution Mass spectroscopy
5. Quantitative assessment of neurotransmitter variation in glaucomatous patients using Triple Quad Mass spectrometry
6. To explore the role of Trace amine receptors in eye
7. Assessment of Beta-adenoceptor and organic anion transporter polymorphism in patients with Open angle glaucoma

Objective 1: Primary metabolite profiling in aqueous humor of glaucomatous patients using NMR

Purpose: Glaucoma is a multifactorial disease, leading to retinal ganglion cell death (RGCs) death, optic neuropathy, and irreversible loss of vision. It is essential to understand the metabolic vulnerability of the disease to identify the key metabolic pathways involved in pathophysiology. This study was conducted for profiling the primary metabolites present in human aqueous humor and their variation in POAG and PACG using proton Nuclear Magnetic Resonance (NMR) spectroscopy.

Methods: This case-control study involved three age-matched groups of patients with primary open angle glaucoma (POAG, n=15), primary angle closure glaucoma (PACG, n=14) and cataract patients (control, n=16). Patients aqueous humor of 70 to 100 µL was collected by paracentesis during trabeculectomy (study group) and cataract surgery (control), snap frozen at -80 °C and subjected for Nuclear magnetic resonance (NMR) analysis. 1 D Proton NMR Spectras were acquired on a 700 MHz spectrometer. The individual metabolites were identified using published literature using human metabolome database and the datasets were analysed using multivariate analysis i.e., Principal Component Analysis (PCA) and Partial lease square discriminant analysis (PLS-DA) using Metaboanalyst software version 5.0.

Results: Baseline Intraocular pressure (IOP), drug treated IOP, and cup-to-disc ratio was significantly elevated in both glaucomatous group versus control. In Untargeted analysis, PCA score plot has shown pattern of group clustering. PLS-DA score plot of 25 selected metabolites had shown the distinct separation between both glaucomatous study groups from control. Quantitative analysis of targeted metabolites attributed a significantly elevated levels of glutamate (POAG, $p<0.001$ and PACG, $p<0.05$), ascorbate (POAG, $p<0.05$) and TCA metabolites (Succinate, α-keto glutarate and citrate) in glaucomatous group as compared to cataract control.

Conclusion: NMR metabolomics of human glaucomatous aqueous humor had shown altered TCA cycle metabolites and elevated glutamate, revealed the excitotoxicity and hypoxia/ ischemia driven altered bioenergetics leading to apoptotic events of RGCs in glaucomatous conditions.

Objective 2: Untargeted metabolomics in glaucomatous patients using high resolution mass spectrometry

Purpose: Glaucoma is a multifactorial disease leading to optic neuropathy and irreversible loss of vision. Understanding the metabolic dysregulation underlying complex pathological mechanisms may help to discover early diagnostic biomarker and fetch new therapeutics for glaucoma. This study aims to compare the alterations in the metabolomic profiles of glaucomatous aqueous humor and plasma with the respective controls.

Methods: This study cohort comprised of primary open angle (POAG), primary angle closure (PACG) glaucomatous groups and age as well as sex-matched cataract control. Aqueous humor (70 to 100µL) and plasma (2mL) samples were collected during trabeculectomy and cataract surgeries, snap frozen at -80°C, later processed and subjected for high resolution mass spectrometry (HRMS) analysis. Spectra was processed and the data was acquired using Xcalibur and Compound Discoverer (v4.1) softwares respectively. Univariate and multivariate statistical analysis were carried out using Metaboanalyst ver 5.0.

Results: Overall 12 and 9 metabolites were found to be significantly altered ($p<0.05$, variable importance of projection >1 and $\log_2$ fold change ≥ 0.58/≤ −0.58) in the aqueous humor of POAG and PACG patients, respectively. Out of these, 6 and 5 metabolites were uniquely associated with POAG and PACG aqueous humor samples, respectively. Plasma metabolomics helped to rule out the metabolites altered in the aqueous humor under the systemic influence. Interestingly, 46.6% and 56% of metabolites found its unique association in the plasma of POAG and PACG, respectively. Furthermore, pathway analysis has revealed altered galactose, amino sugar and nucleotide sugar metabolism in the aqueous humor of PACG patients. Altered TCA cycle, spingolipid, glutamate and glutamine metabolism are the pathways with significant impact in POAG.

Conclusion: This study provides newer insights in to the distinct localized metabolites of aqueous humor differentially regulated in the POAG and PACG from their respective alterations in the systemic circulations.

Objective 3: Profiling proteomic variation in glaucomatous patients using High Resolution Mass spectroscopy

Purpose: Glaucoma is a multifactorial, irreversible optic neuropathy and the second leading cause of blindness worldwide with no cure to date. Therefore, understanding the proteomic profiles of both aqueous humor and plasma of glaucoma patients becomes an essential component to find out the diagnostic biomarkers and thus identifying novel therapeutic targets to prevent retinal ganglion cell (RGC) death. Therefore, it is essential to study the alteration in the proteomic profiles of primary open-angle (POAG) and angle-closure (PACG) glaucomatous patients versus cataract control.

Methods: Total protein concentration of aqueous humor and plasma of all the samples was determined by Bradford assay followed by, standard trypsinization and clean-up protocols subjected for proteomic analysis using nano liquid chromatography system (Easy n-LC, Thermo Scientific, USA) coupled to Orbitrap Fusion mass spectrometer (Thermo Scientific, USA). Data analysis was carried out using the database searching algorithm of the Proteome Discoverer software (version 2.0, Thermo Scientific, USA) using the SEQUEST-HT search engine. Gene ontology (GO) analysis of unique proteins particular to POAG and PACG group was carried out using STRING online web tool. Univariate and multivariate analysis of common proteins among study groups were carried out using Metaboanalyst (ver 5.0).

Results: Total protein concentration was found to be significantly elevated in the POAG aqueous humor as compared to cataract controls. Total of 2582 and 4750 proteins were identified in the aqueous humor and plasma of the study population. The GO analysis of unique proteins of POAG and PACG revealed the proteins related the Parkinson's disease with necroptosis and dysfunction in dopaminergic synaptic dysfunction respectively. On analysing the 107 common master proteins, overall 14 and 9 proteins were found to be significantly altered ($p<0.05$, variable importance of projection >1 and $\log_2$ fold change $\geq 0.58/\leq -0.58$) in the aqueous humor of POAG and PACG patients, respectively. Out of these, 11 and 6 master proteins were uniquely associated with POAG and PACG aqueous humor samples, respectively. Plasma proteomics helped to rule out the proteins altered in the aqueous humor under the systemic influence.

Conclusion: In POAG, glycoprotein elevation, lipoprotein, and Wnt signalling cascade dysregulation were the key localized changes. Fibrotic events locally associated with the pathology of PACG. Altered lipoprotein metabolism and elevated serum amyloid protein are the key systemic events of glaucomatous conditions.

Objective 4: Profiling Lipidomic variation in glaucomatous patients using High Resolution Mass spectroscopy

Purpose: To determine the alteration in the aqueous humor and plasma lipidomic profiles of primary open angle (POAG) and angle closure glaucomatous (PACG) patients with respect to the cataract control. Understanding the lipidomic alterations may expect to delineate the underlying pathological lipid signalling mechanisms involved in glaucoma.

Method: Human aqueous humor, plasma and serum samples were collected from both of glaucomatous patient groups and from cataract controls. Routine serum lipid profiles were carried out using biochemical based analyser. Lipids were extracted from the plasma and aqueous humor and subjected for high resolution-based mass spectrometer (HRMS) analysis. Lipid identification and relative quantification was carried out using LipidSearch™ ver 4.1. Statistical analysis was carried out using Metaboanalyst ver5.0.

Result: Combined univariate and multivariate analysis has shown the significantly elevated lipid species localized to aqueous humor. This study shown majority of the phospholipid and sphingomyelin species were found elevated in the aqueous humor of both POAG and PACG. Di- and Triglycerides were found declined in both glaucomatous conditions. Clinical serum lipid profiles were found insignificant among the study groups versus control.

Conclusion: This study demonstrated an altered lipidome in aqueous humor and plasma during glaucomatous conditions. Functional role of individual lipids species and their involvement in offering trabecular meshwork outflow resistance and mechano-signalling must be understood further.

Objective 5: Quantitative assessment of neurotransmitter variation in glaucomatous patients using Triple Quadruple Mass spectrometry

Purpose: Neurotransmitters (NTs) are the key mediators of performing essential ocular functions like, processing the visual functions of the retina, maintaining homeostasis of aqueous humor and regulating ocular blood flow. This study aims to determine variations in the levels of L-glutamate and γ- aminobutyric acid (GABA), histaminergic, adrenergic, cholinergic, and serotonergic NTs in primary glaucomatous versus cataract patients.

Methods: This case-control study involved three age-matched groups of patients with primary open angle glaucoma (POAG, n=14), primary angle closure glaucoma (PACG, n=21) and cataract patients (control, n=19). Patient's aqueous humor and plasma were collected, snap frozen at -80°C and were subjected to ultrasensitive liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis for the quantification of NTs.

Results: Baseline intraocular pressure and cup-to-disc ratio was found to be significantly elevated in both POAG and PACG as compared to the control group. In aqueous humor, histamine was found to be significantly elevated (5-fold, $p<0.0001$) whereas 1-methyl histamine was significantly decreased ($p<0.05$) in POAG as compared to the control. Significant increase in L-glutamate and GABA has been observed among both the glaucomatous patient groups as compared to the cataract control. Adrenaline was found to be elevated only in the PACG group (2.7-fold, $p<0.05$). No statistically significant difference was observed among the plasma NT levels between the groups.

Conclusions: This study demonstrated the prominent role of histaminergic system apart from autonomic mechanisms in the progression of glaucoma. Elevated L-glutamate and GABA could be due to the retinal ganglionic cell death. Further studies are required to evaluate the effects of histamine on Müller cell dysfunction.

Object 6: To explore the role of Trace amine receptors in eye

Purpose: Trace amine associated receptors (TAAR) are the G- protein coupled receptors, the emerging potential pharmacological target for the various neurological and immunological disorders (Freyberg Z et al. 2020). This study explored its role in eye to understand its pharmacological effect.

Methods: Gene expression profiles of TAAR-1 receptor in various rat ocular structures were carried out. Topical β-phenyl ethyl amine (β-PEA) eye drop formulation was prepared for the pharmacological evaluation of β-PEA in rabbit eyes. Molecular Docking studies, Ocular pharmacokinetics of 0.25% β-PEA in rabbit eyes, Liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis for determining the β PEA levels in rabbits and human aqueous humor were carried out.

Results: TAAR-1 receptors were found to be expressed well in iris-ciliary body, optic nerve and lens, poorly expressed in cornea and not expressed in the retina of rats. Maximum mydriatic response was observed at 15 min following single instillation of 0.25% β-PEA eye drops. No significant changes in the IOP has been observed in any of the concentrations on single- and multiple-instillations of β-PEA. Rabbit eyes were found with presence of light reflex. Aqueous humor of POAG Patients found insignificantly elevated β-PEA levels as compared to the cataract control.

Conclusion: β-PEA is capable of causing adrenergic response through TAAR-1 pathway. This observation for the first time explains the multiplicity of pathways involved in increased IOP where in POAG. Moreover, this observation gives the possibility of developing topical TAAR antagonists for effective control of IOP.

Objective 7: Assessment of Beta-adenoceptor and organic anion transporter polymorphism in patients with Open angle glaucoma

Purpose: Emergence of Adrenergic β2 receptor (ADRB2) blockers has evolved as an effective treatment for glaucoma and the subsequent development of prostaglandin analogues further extended the therapeutic options. Therefore, understanding the genetic polymorphism in the ADRB2 and drug transporters involved in the ocular kinetics of topically applied prostaglandin analogues like solute carrier organic anion transporter family member 2A1 (SLO2A1) is expected to address variations in the therapeutic response to these agents in patients due to altered pharmacodynamics and kinetics.

Methodology: Blood samples (2mL) were collected and DNA was extracted using phenol-chloroform procedure. Group of primary open angle glaucoma (POAG) patients (n=77) was compared with the non-glaucomatous controls (n=55) for the presence of polymorphism rs1042713 (Arg16Gly, A>G) and rs1042714 (Gln27Glu, C>G) in the ADRB2 gene and rs34550074 (Ala396Thr, A>G) in SLCO2A1 gene using Sanger sequencing. Hardy- Weinberg equation was used to derive the frequencies of expected genotype from wild-type allele and statistical analysis was carried out using the Chi-square test.

Result: A total of 137 patients were enrolled in this study. POAG group was found to have a significantly elevated IOP at base line and during glaucoma therapy. Distribution of genotype for the studied polymorphism was found to have a Hardy Weinberg equilibrium. GA genotype of rs1042713 and GG genotype of rs1042714 were positively correlated with the POAG whereas codon of rs34550074 was not found to have association with POAG.

Conclusion: This study reveals the association of ADRB2 gene with open angle glaucoma whereas variation in SLCO2A1 did not show any significance.

Over all summary and conclusion

Glaucoma is a disorder eventually leading to the retinal ganglion cell (RGC) death with irreversible vision loss. As per National Health Portal of India 2021, glaucoma is the second leading cause of blindness in India as well as worldwide. Due to its multifactorial nature, occurrence of pathophysiological features is poorly understood, and hence no curative treatment is available to reverse the vision loss. Among available anti-glaucoma medications, topically administered prostaglandin analogues and beta blockers remain as first line of choice for glaucoma treatment. Progressing neurodegeneration of the retina even after effective drug therapy with available antiglaucoma medications pose challenge for treating Ophthalmologists. To understand their inter individual variability to drug response a rational multi-dimensional approach like Omics are required to get more insights about the disease process and to look for the possibility of increasing more molecular pathways for therapeutic benefits. Therefore, the primary aim of the present study was to identify both the localized and systemic alterations in multi-omics profiles associated with POAG and primary angle closure glaucoma (PACG), with the secondary aim to understand the association of SNPs presents in ADRB2 and OATP2A1 in patients with POAG.

To understand the metabolic profile in aqueous humor of glaucoma patients, NMR metabolomics was used for quick screening strategy. Aqueous humor of POAG, PACG and control were subjected for NMR based prediction. In untargeted analysis, PCA score plot has shown pattern of group clustering. PLS-DA score plot of 25 selected metabolites had shown the distinct separation between both glaucomatous study groups from control. Quantitative analysis of targeted metabolites attributed a significantly elevated levels of glutamate (POAG, $p<0.001$ and PACG, $p<0.05$), ascorbate (POAG, $p<0.05$) and TCA metabolites (Succinate, α-keto glutarate and citrate) in glaucomatous group as compared to cataract control.

This study revealed the altered TCA cycle metabolites and elevated glutamate. This alteration might be responsible for the hypoxia/ ischemia induced altered bioenergetics and apoptotic events of RGCs reflected in aqueous humor. However, using this technique was inadequate to explain the changes in other metabolites in the pathology of glaucoma. Therefore, to achieve the wider perspective of metabolomics further analysis was done using Mass spectrometry.

High resolution mass spectrometry (HRMS) was utilized for the global assessment of metabolite profile in the aqueous humor to assess altered metabolic pathways associated with

POAG and PACG. Both aqueous humor and plasma of the glaucomatous patients and cataract controls were subjected for HRMS analysis in positive and negative ion mode for screening both polar and non-polar metabolites. Overall 12 and 9 metabolites were found to be significantly altered ($p<0.05$, variable importance of projection >1 and log2 fold change ≥ 0.58/≤ −0.58) in the aqueous humor of POAG and PACG patients, respectively. Out of these, 6 and 5 metabolites were uniquely associated with POAG and PACG aqueous humor samples, respectively. Plasma metabolomics helped to rule out the metabolites altered in the aqueous humor under the systemic influence. Interestingly, 46.6% and 56% of metabolites found its unique association in the plasma of POAG and PACG, respectively. Furthermore, pathway analysis revealed altered galactose, amino sugar and nucleotide sugar metabolism in the aqueous humor of PACG patients. Altered TCA cycle, spingolipid, glutamate and glutamine metabolism are the pathways with significant impact in POAG. The findings of this study indicated the mitochondrial dysfunction, altered sugar metabolism, oxidative and metabolic stress were identified as localized mechanisms involved in PACG. However, in the plasma of PACG patients, lysophospholipid and TCA cycle metabolism were majorly altered which might have been contributed for the disease pathology. Altered TCA cycle metabolism and mitochondrial membrane transport in addition to life style factors might have also contribute to the localized changes in POAG. Systemic abnormalities including defects in primary bile acid biosynthesis, sphingolipid metabolism have also been found to contribute towards the progression of POAG.

One of the interesting findings of untargeted metabolomics through HRMS highlighted the local and systemic alterations in the endogenous monoamine metabolism which includes neurotransmitters and trace amines. Therefore, the study was further extended to quantify their absolute levels from the aqueous humor and plasma of POAG, PACG and cataract controls. Interestingly, this study observed 5-fold elevation of histamine and 2.5 fold elevation of adrenaline levels in the aqueous humor of POAG and PACG aqueous humor respectively. Localized elevation of glutamine and GABA were found in both of the studied glaucomatous group. No significant changes were associated with any of the studied systemic neurotransmitter levels. This highlighted the possibility of histaminergic system in glaucoma progression. To explore other metabolites like endogenous trace amine (beta phenyl ethyl amine, β PEA), further experiments were carried out to study the effect of β PEA in normal rabbit eyes. This experiment clearly showed that PEA is capable of causing adrenergic type of response through TAAR-1 pathway. This observation for the first time explains the multiplicity of pathways involved in increased IOP where in POAG. Moreover, this observation highlights the

possibility of developing topical TAAR antagonists for the effective control of IOP. Further studies in this direction are essential to develop topical TAAR antagonists and H3 receptor blockers for effective control of IOP with or without existing antiglaucoma medications.

To explore the altered pathways in the pathophysiology of glaucoma, this study was further extended to LC-MS (HRMS) based omics-approaches including proteomics and lipidomics. Both aqueous humor and plasma of the glaucomatous patients and cataract controls were subjected for HRMS analysis for analysing the changes in proteomic and lipidomic profiles. This analysis revealed significantly elevated total protein concentration in the POAG aqueous humor as compared to cataract controls. Total of 2582 and 4750 proteins were identified in the aqueous humor and plasma of the study population. The GO analysis of unique proteins of POAG and PACG revealed the presence of proteins related to the Parkinson's disease (neurodegenerative) with necroptosis and dopaminergic synaptic dysfunction. On analysing the 107 common master proteins, overall 14 and 9 proteins were found to be significantly altered ($p<0.05$, variable importance of projection >1 and log2 fold change ≥ 0.58/≤ −0.58) in the aqueous humor of POAG and PACG patients, respectively. Out of these, 11 and 6 master proteins were uniquely associated with POAG and PACG aqueous humor samples, respectively. Altered proteins localized to the aqueous humor of POAG related to neurodegeneration, necroptosis, elevated O-glycosylated protein (mucin like protein 1), lipoprotein alteration and Wnt signalling dysregulation. Similarly, in PACG, altered proteins related to the fibrotic events were observed. Proteins identified in the plasma proteomics revealed the altered lipoprotein metabolism and elevated serum amyloid protein may be involved in the pathophysiology of POAG and PACG respectively.

In lipidomic analysis, clinical serum lipid profiles were found insignificant among the study groups versus control. However, combined univariate and multivariate analysis of HRMS data sets revealed the alterations were particular among the lipid species and their respective lipid classes in glaucoma. Majority of the phospholipid and sphingomyelin species were found elevated in the aqueous humors of both POAG and PACG, whereas Di- and Triglycerides were found to decline in both glaucomatous conditions. This indicates the possibility of membrane disruption due to increased exicitotoxicity. Present study demonstrated an altered lipidome in aqueous humor and plasma in glaucomatous conditions. However, functional role of individual lipid species and their involvement in offering trabecular meshwork outflow resistance and mechano-signalling is yet to be understood.

To delineate the existence of genetic polymorphism in the adrenergic beta-2-receptor (ADRB2 -rs1042713, rs1042714) and organic anion transporter (SLCO2A1- rs34550074) studies were carried out in the genes of patients with POAG and compared with the non-glaucomatous control. A total of 137 patients were enrolled in this study. Distribution of genotype for the studied polymorphism was found to have a Hardy Weinberg equilibrium. GA genotype of rs1042713 and GG genotype of rs1042714 were positively correlated with the POAG whereas codon of rs34550074 was not found to have any association with POAG. The results from the study showed association of ADRB2 gene with open angle glaucoma whereas variation in SLCO2A1 did not show any significance.

To conclude, this study was initiated with an aim to identify the localized and systemic alterations in metabolomic, lipidomic and proteomic profiles in POAG and PACG patients and was compared to non-glaucomatous controls. Quantitative analysis of targeted metabolites using NMR revealed significantly elevated levels of glutamate, ascorbate and TCA metabolites (Succinate, α-keto glutarate and citrate) in glaucomatous group as compared to cataract control. Studies using HRMS revealed the alteration in omics studies and their metabolic pathway analysis indicated occurrence of altered galactose, amino sugar and nucleotide sugar metabolism in the aqueous humor of PACG patients. Altered TCA cycle, spingolipid, glutamate and glutamine metabolism are associated with POAG. Analysing biologically relevant amines indicated the 5-fold elevation of histamine and 2.5fold elevation of adrenaline levels in the aqueous humor of POAG and PACG respectively. Elevated beta phenyl ethyl amine (Trace amines) in the aqueous humor of POAG group was subsequently demonstrated in the animal model about its propensity to cause mydriasis. These findings highlight the possibility their involvement in the glaucoma and enabling the utilization of pharmacological interventions such as antagonists of histaminergic and TAAR receptors in the treatment of glaucoma.

Although this study demonstrated an altered lipidome in aqueous humor and plasma in glaucomatous conditions, functional role of individual lipid species and their involvement in offering trabecular meshwork outflow resistance and mechano-signalling is yet to be understood. Genetic polymorphism studies conducted using selective SNPs revealed the possibility of variation in the adrenergic beta2 receptors in the patients with POAG. However, further studies are required to prove the hypothesis in the patients with therapeutic failure using beta-blockers like timolol. The multi-Omics approaches adopted in this study indicated

many significant findings having immense importance in understanding the pathophysiology of glaucoma and other possibilities to develop newer pharmacological interventions.

Limitations:

Due to the COVID-19 pandemic, limited number of trabeculectomy and cataract surgeries were conducted which restricted the sample size for the analysis carried out and their interpretation in the study. Extensive studies correlating with the failure of beta-blocker therapy could not be ascertained in the genetic polymorphism studied on ADRB2 and SLCO2A1 genes. Since the nature of the conducted studies on multi-omics were exploratory, further studies are required to validate the findings using larger datasets. Effect of topical anti-glaucoma medications on the multi-omics profile of glaucomatous aqueous humors need to be studied to rule out their influence in the identified molecular pathways. However, considering the limitation of time and COVID-19 pandemic, this study was not possible to comprehend within the given timeframe.

Future directions:

Future studies are required to integrate the multi-omic variations observed in this study to provide the overview of alterations in the system biology during glaucoma. Extensive studies are recommended with larger sample size and on targeted pathways to explore/validate novel biomarkers for glaucoma. These markers would be of benefit in individualizing and rationalizing pharmacotherapy for glaucoma. Moreover, to understand the genetic polymorphism in drug response studies with increased number of subjects may be required with other SNPs. Patients not responding to beta blockers and prostaglandins must be isolated to prove the aforesaid polymorphism.

Impact of the research in the advancement of knowledge or benefit to mankind

1. Altered bioenergetics, excitotoxicity and apoptotic events of RGCs reflected in aqueous humor of glaucomatous patients.

2. Abnormal fibrotic events, mitochondrial dysfunction, altered sugar metabolism, oxidative metabolic stress were identified as localized mechanisms involved in PACG.

3. This study highlighted the importance for the utilization of pharmacological interventions such as antagonists of histaminergic and TAAR receptors in the treatment of glaucoma.

4. Neurodegeneration, necroptosis, elevated O-glycosylated protein (mucin like protein 1), lipoprotein alteration, Wnt signalling dysregulation, disturbance in mitochondrial membrane transport in addition to life style factors were involved in the pathophysiology of POAG.

5. This study has shown the dysfunction in phospholipid, sphingomyelin and Glyceride metabolism in both POAG and PACG.

6. This study has shown the association of ADRB2 gene with open angle glaucoma Indian population whereas variation in SLCO2A1 did not show any significance.

7. Amalgamation of the interesting results obtained from the different levels of multi-omic data sets from this study may have a huge impact on the optimization of personalized medicine for glaucoma treatment in Indian population.

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