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Correlation Between Myopia And Some Immunological Aspects In Primary Open Angle Glaucoma Iraqi Patients

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Abstract

The objective(s) : Estimate the correlation between myopia and TGF-β1, TGF-β2, TNFα, CD4+, CD8+ markers and FoxP3 in Primary open angle glaucoma , evaluation the role of some cytokines (TGF-β1, TGF-β2, and TNFα), CD4+, CD8+ markers and FoxP3 in pathogenesis of Glaucoma. Materials and Methods: Blood samples from patients with Primary Open Angle Glaucoma (POAG) were taken at Baghdad's Ibn Al-Haitham Teaching Eye Hospital. Also, the samples have been taken from 75 unrelated Iraqi POAG patients (49 males and 26 females) and 75 unrelated healthy people (50 males and 25 females) within the age of 40 –70 years through the period from April to September 2021. For detecting CD8, CD4, D4/CD8 ratio, FOXP3, TNF-α, TGF-β2, TGF-β1, ELISA and flowcytometry approaches are utilized. The results: the age groups of 50-59 and 60-69 years scored highest percentage in patients than controls ($p<0.05$). There are low levels of CD4, FoxP3 and Ratio and high levels of CD8, TGF-β1, TNF-α, and TGF-β2 in patients than controls with significant different ($p<0.05$). The TGF-β1, TNFα, and TGF-β2 were scored highest sensitivity (96%, 97% and 92%) respectively than others parameters with significant different ($p<0.05$). Conclusions; Glaucoma is increased with age progression. TNF-α, TGF-β1 and TGF-β2 parameters can be used as predictors biomarkers for Glaucoma diagnosis.

Keywords; Glaucoma, T regulatory, TGF-β1 and TGF-β2, FOXP3, CD4,CD8, TNF-α, myopia .

Introduction

Glaucoma can be defined as one of the neurodegenerative conditions affecting the eyes and is linked to increased intraocular pressure (IOP). If left untreated, patients' visual field might be lost over time, and they can eventually lose their sight entirely. It is the world's second biggest blindness cause (Bezuidenhout and Schellack, 2015). Adults and individuals over the age of 60 are particularly susceptible to this condition. Optic nerve deterioration takes place in the Optic Disc (OD) region referred to as Optic Nerve Head (ONH). Glaucoma cannot be cured, and the damage it causes is largely irreparable. The only choices offered to patients are strategies to reduce the disease's course. The effectiveness of all treatments depends on a timely diagnosis of the condition (Abdullah et al., 2021). Pathogenesis, time of onset, etiology, and anatomy of the anterior chamber angle are all used to classify glaucoma (Bowling, 2016).

Glaucoma is expected to impact about 80 million individuals by 2020, and 111.80 million individual by 2040, with a greater number of people living in African and Asia (Tham et al., 2014). Glaucoma is expected to be present in between (3 and 5)% of white population, also between (5 and 7)% of black population in South Africa (Bezuidenhout and Schellack, 2015). POAG is linked to age, and individuals of African descent have a

high risk to develop it compared to non-Africans (Broman and Quigley, 2006). Glaucoma has a significant economic impact because it impairs both the nation's and individual's productivity. Almost all population is ignorant of their glaucoma state, making glaucoma care a problem for eye health providers (Abdullah et al., 2021). PCG-causing variations have been discovered in 10 Arab nations, with the majority of them being found in CYB1P1 gene. Furthermore, genotype-phenotype associations in Arab patients with PCG appear to be unique (Jemmeih et al., 2021).

The eye is known to be an immune-privileged site. In the eyes, the active local immunosuppression is specified via the blood-aqueous and blood-retina barriers, the pigment epithelial cells' distinctive characteristic, and local synthesis of immunosuppressive cytokines and neuro-peptides, among other things (Stein-Streilein, 2008). Inflammation, such as that which takes place in response to pathological or surgical, carries the risk of vision impairment. Because immune responses are such an important part of the repair response, the eye has evolved its own methods for delivering immune responses to injury in avascular areas (Menko and Stepp, 2021).

The retina's activated glial cells in experimental glaucoma models express MHC-II proteins and release pro-inflammatory chemokines and cytokines such as TNF-α (Baudouin et al., 2020). Aqueous TGF-β has a high diagnostic performance in identifying glaucoma sub-types and can be prospective glaucoma biomarkers (Igarashi et al., 2021). The activation regarding Fas-FasL signaling causes immune cells which enter the eye as a response to injury to infection to undergo apoptosis without producing tissue damage or inflammation (Jiang et al., 2020).

The introduction of antigenic material into the eye causes immune deviation or inhibition of T cell-mediated immunity, resulting in peripheral immune tolerance to antigens, an approach known as Anterior Chamber Associated Immune Deviation (Moalem et al., 1999). Glaucoma patients had a lower number of Treg, and their CD4+ T cells had a higher stimulation response, which included enhanced proliferation and pro-inflammatory cytokine secretion. CD3+CD8+ lymphocyte frequency values were shown to be higher in patients with primary open-angle glaucoma (POAG) and normal tension glaucoma (NTG), with CD8+HLA-DR+ lymphocytes being more prominent in NTG. Increased expression regarding the soluble IL2 receptor, a marker of the T cell activation, accompanied this (Jiang et al., 2020). Latest research also suggests that systemic adaptive immune responses play a key role in the glaucoma's pathogenesis. In the glaucoma patient's sera, complex patterns related to retinal proteins as well as autoantibodies against retinal specific antigens were discovered (Beutgen et al., 2019). The purpose of this work is to determine the role of certain cytokines, FoxP3 Treg and CD markers in the glaucoma's pathogenesis.

Materials and methods

Samples collection

The collection of blood samples and practical work for the presented work lasted from April to September 2021. Also, the samples have been taken from a total of 75 unrelated Iraqi patients with POAG (26 females and 49 males) and a total of 75 unrelated healthy individuals (25 females and 50 males) serving as controls in this work. All controls and patients are of the same ethnicity, have no ocular or systemic disease, and are between the ages of 40 and 70. Patients experiencing POAG have been recruited from Baghdad's Ibn Al-Haitham

Teaching Eye Hospital. In addition, the patients clinically diagnosed via the Glaucoma Department's consulting medical team. The following are the diagnostic criteria used for diagnosing POAG with myopia: Patients' age, a high IOP of more than 21 mmHg, a high cup-to-disk (c/d) ratio, and an expanded cornea of more than 11 mm. A venous blood sample of 5 milliliters was obtained and divided into 2 parts. The first part (3 ml) has been placed in gel tubes and allowed to sit for 15 mins at room temperature before being centrifuged at 2000 rpm for a period of 15 mins to obtain sera, which were after that stored at a temperature of -20 Celsius till utilized for cytokine (TGF-β1, TNF-α, and TGF-β2) measurement via ELISA method. The second part (1 ml) was placed in EDTA tubes for flow cytometry analysis of CD markers, CD4/CD8 Ratio, and FOXP3treg.

Detection of study parameters

A- Detection of CD markers, FOXP3 Tregs and CD4/CD8 Ratio by flow cytometry technique.

In this study, Immunophenotyping CD4+, CD8+, CD4/CD8 ratio and foxp3+ were investigated by using fully equipped desktop three–color flowcytometry (BD FACS, USA).

- CD8-APC H7(mouse IgG), (catalog no. E-AB-F1110S, Elabscience, USA)
- CD4-FITC (mouse IgG), (catalog no. E-AB-F1231C, Elabscience, USA)
- FOXP3-PE (mouse IgG), (catalog no. IC8970P, R&D, USA
- Flow Cytometry FOXP3 staining buffer (120ml), (R&D, USA)
- Flow cytometry Staining Buffer (50ml), (BD FACS., USA)

B- Detection of TNF-α, TGF-β1 and TGF-β2 by ELISA technique

Serum levels of cytokines (TGF-β1, TNF-α, and TGF-β2) were quantified by sandwich ELISA (Humareader HS, Germany) technique using the commercially available kit (KOMA BIOTECH. Korea). The concentrations of the various cytokines detected were in pg/ml. measure observance at 450 nm (as the manufacturer's protocols).

Statistical analysis

The Chi-square (X2) test was used for comparing percentages in the present work's data. (Mean ± St. Error) was used to characterize numerical data. For comparing two numeric variables, the Student T test is utilized. The F test was also employed to assess the association between study parameters and age groups in this investigation. The test was run using a significance level of no more than (p=0.05). Present data was analyzed using (Graph pad prism v.6 and SPSS v.22) programs.

Results

Age groups and gender within study groups

Data of current study show the 50-59 and 60-69 age groups scored highest percentage in myopia groups and healthy compared to others age groups, where the 50-59 age group scored percentage (22.9%, 25.9%, and 40.0%) , while 60-69 group scored percentage (54.2%, 51.9%, and 37.3%) for patients with myopia , patients

without myopia, and healthy respectively. There is significant different ($p<0.05$) among age group and study groups (table 1).

Respect to gender, our results reveal the males scored high percentage (64.6%, 66.7%, and 66.7%) for for patients with myopia , patients without myopia, and healthy respectively than females with non significant different ($p>0.05$) (table 1).

Table 1; comparative age groups and gender within healthy and myopia group in Glaucoma patients

			Myopia			Total	p value
			Yes	No	Control		
Age groups (years)	40-49	n	5	2	15	22	0.034*
		%	10.4%	7.4%	20.0%	14.7%	
	50-59	n	11	7	30	48	
		%	22.9%	25.9%	40.0%	32.0%	
	60-69	n	26	14	28	68	
		%	54.2%	51.9%	37.3%	45.3%	
	>70	n	6	4	2	12	
		%	12.5%	14.8%	2.7%	8.0%	
Gender	Males	n	31	18	50	99	0.96 (N.S)
		%	64.6%	66.7%	66.7%	66.0%	
	Females	n	17	9	25	51	
		%	35.4%	33.3%	33.3%	34.0%	

Chi-square test. * Significant different, N.S = Non significant($P>0.05$). POAG: primary open angle glaucoma.

Mean levels of immunological parameters within study group

Our study show the CD4 parameter scored highest mean level at healthy (56.86 ± 0.89) and lowest at patients without myopia (51.64 ± 2.27) with significant different between healthy and patients without myopia and between patients with myopia and patients without myopia ($p<0.05$), but non significant between patients with myopia and healthy groups ($p>0.05$, LSD=2.88). based on FoxP3, the mean level of it was highest at healthy (85.53 ± 1.43) and lowest at patients without myopia (66.65 ± 5.28) with significant different between healthy and patients without myopia and between patients with myopia and patients without myopia ($p<0.05$), but non significant between patients with myopia and healthy groups ($p>0.05$, LSD=7.12). There is no significant different among study groups depend on CD8 and ratio ($p>0.05$) (table 2).

Table 2; mean levels of immunological parameters within healthy and myopia group.

Myopia		N	Mean	Std. Error	Statistics
CD4	Yes	48	55.07 [a]	1.18	P<0.05*

	No	27	51.64 [b]	2.27	LSD=2.88
	Healthy	75	56.86 [a]	0.89	
CD8	Yes	48	35.78	1.33	
	No	27	35.79	2.54	
	Healthy	75	32.83	1.03	P>0.05
FoxP3	Yes	48	69.92 [b]	3.66	
	No	27	66.65 [b]	5.28	P<0.001***
	Healthy	75	85.53 [a]	1.43	LSD=7.12
CD4/CD8Ratio	Yes	48	1.62	0.09	
	No	27	1.59	0.16	
	Healthy	75	1.87	0.08	P>0.05

LSD= least significant different , Small different letters refer to significant different

Mean levels of TNF-a, TGF- β 1 and TGF- β 2 parameters within study group

The conducted study show the TNF_a , TGF_ β 1, and TGF_ β 2 parameters scored highest mean level at patients with myopia (143.66 ± 6.10, 388.60 ± 25.31, and 331.28 ± 21.76) respectively, and lowest at healthy (71.52 ± 2.07, 147.45 ± 4.82, and 150.28 ± 5.22) respectively with significant different among groups for all parameters (p<0.05) (table 3).

Table 3; mean levels of TNF-a, TGF- β 1 and TGF- β 2 parameters within healthy and myopia group.

Myopia		N	Mean	Std. Error	Statistics
TNF_a	Yes	48	143.66 [a]	6.10	
	No	27	113.64 [b]	8.37	P<0.001***
	Healthy	75	71.52 [c]	2.07	LSD=15.21
TGF_β1	Yes	48	388.60 [a]	26.70	
	No	27	329.50 [b]	25.31	P<0.001***
	Healthy	75	147.45 [c]	4.82	LSD=53.61
TGF_β2	Yes	48	331.28 [a]	21.76	
	No	27	281.39 [b]	16.21	P<0.001***
	Healthy	75	150.28 [c]	5.22	LSD=40.51

Receiver operator characteristic curve of immunological parameters

Result of conducted study shows the highest sensitivity was for TNF_a (96%), TGFβ_1 (97%), and TGFβ_2 (92%), than others parameters . Based on specificity, the highest specificity scored at was for CD4 (58%), CD8 (52%) , FOXP3+ Treg (53%) and Ratio (56%) than others parameters with significant different (p<0.05) (figure 1).

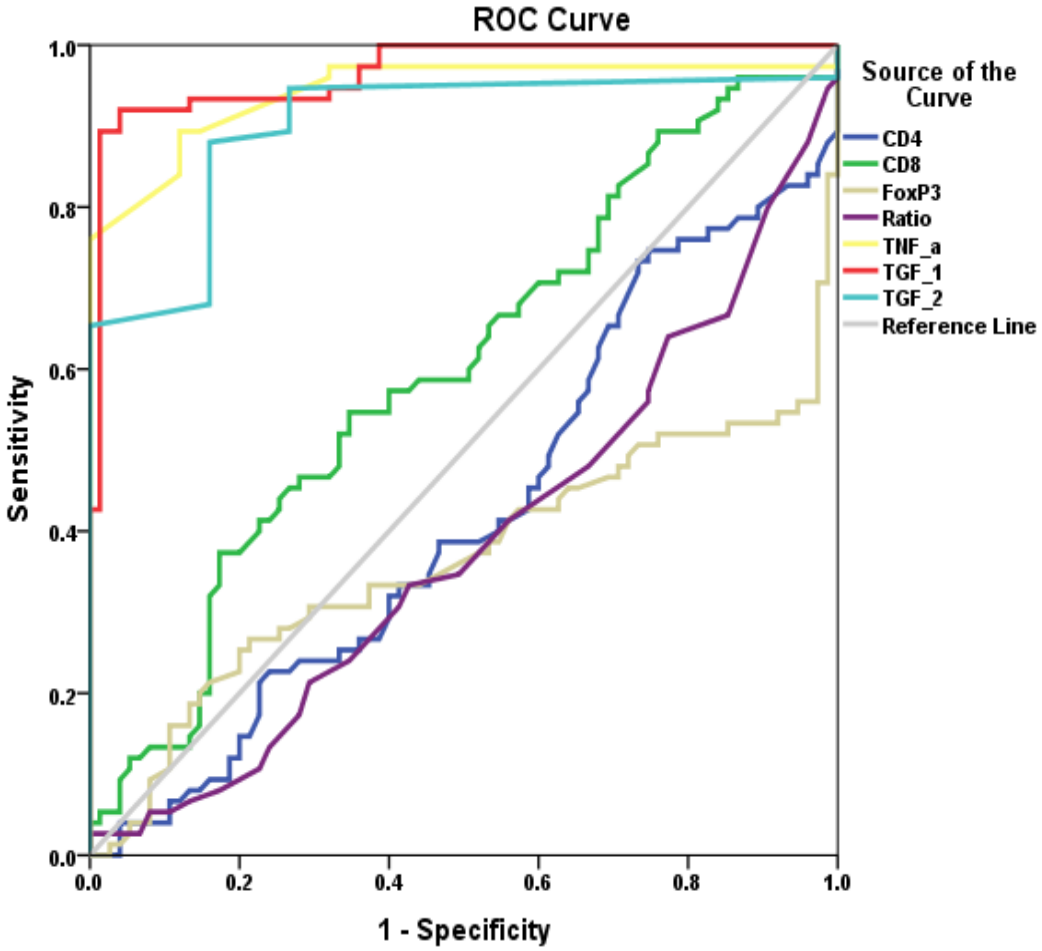

Figure 1; AUC, sensitivity and specificity of immunological parameters .

Discussion

In current study, we observed myopia disease is increased with peoples more than 40 years and that not compatible to results Pan et al., (2015) that showed the Myopia was found to be most prevalent (96.5%; 95% confidence interval, 96.3 to 96.8) in peoples aged 19 years. Ruan et al., (2019) showed high prevalence of myopia in peoples more than 50 years and these results compatible to our results. Risk factors include age and frailty, gender, myopia, genetics, family history, smoking, race, systemic hypotension and hypertension, vasospasm, use of systemic or topical steroids, migraine, obstructive sleep apnea syndrome, and most significantly, increased intraocular pressure (IOP) (Imrie and Tatham, 2016). Study results indicate that long-term exposures to particulate matter (PM2.5) and ozone (O3) might be important environmental risk factors of myopia in the elderly, and suggest that more efforts should be taken to reduce airborne PM2.5 and O3 levels to protect vision health (Ruan et al., 2019). From study results, reporters can conclude that within our sample of children, increased computer use is associated with myopia development (Enthoven et al., 2020). Hopf et al., (2020) revealed the prevalence of myopic maculopathy in the German population was 0.5%, and 10% in high myopic participants, aged 35–74 years. Risk factors for high myopia at adult age are a young age of onset and a fast progression rate during childhood (Koh et al., 2016). Around 90% of all myopic individuals appear to be

stable at the age of 21 years, and nearly all by the age of 24 years (COMET Group, 2013) and this study contrast to our study. Polling et al., (2021) concluded the trajectories of the natural course of myopia progression may serve as a guide for myopia management in European children, spherical equivalent of refraction (SER) at 10 years is an important prognostic indicator and will help determine treatment intensity.

Respect to gender, our results showed there is no significant different between study groups and gender ($p>0.05$). Although the patterns of distribution of refractive errors in patients (males 30% and females 70%) aged 6 to 85 years corroborates previous findings, myopia and hyperopia peak, as well as severity of astigmatism were unique to these study (Wajuihian and Mashige, 2021). Males were significantly more myopic and this finding agrees with several studies (Gomez-Salazar et al., 2017), where these results compatible to our results that showed the males scored high percentage in peoples with myopia. It is widely regarded that myopia occurs more often in girls than in boys, especially in older children. Czepita et al., (2019) also observed a higher occurrence of myopia in girls aged 9 to 16 years. It is widely accepted that there are three possibilities for gender differences. The first is that the differences are biologically determined. The second possibility is that they are socially/behaviorally determined (extensive use of the eyes in reading or the use of mobiles and computers (Czepita et al., 2019).

Our study revealed low levels of CD4 marker in patients with glaucoma than healthy. The high levels of CD4 in glaucoma patients with myopia is more than patients without myopia due increased inflammatory process in patients with myopia, where the evidence showed that inflammation plays a crucial role in the development of myopia (Lin et al., 2016). The ocular surface immune response involves both innate and adaptive mechanisms (Schaumburg et al., 2011). It occurs at the corneal surface, in ocular tissues and regional lymph nodes, and involves T helper (T_H) cells, memory T cells, and regulatory T cells (Tregs) (Perez et al., 2016). It is a complex and tightly regulated process that is designed to protect and defend the ocular surface but, when dysregulated, can lead to eye diseases (Pflugfelder and de Paiva, 2017). A variety of immune cells reside at the ocular surface: natural killer immune cells, dendritic cells (which are the primary antigen-presenting cells , macrophages, gamma delta (γδ) cells, and, to a limited extent, alpha beta (αβ) T cells ($CD4^+$ and $CD8^+$) (Pflugfelder and de Paiva, 2017). Bell et al., (2017) show high levels of CD4 in glaucoma patients than controls and that not compatible to our results. Studies of experimental models have provided data supportive of stimulated T-cell responses with neurodegenerative potential (Chen et al., 2018). Yang et al., (2019) mentioned the patients with glaucoma exhibited a trend of decreased frequency of Treg and their CD4+ T cells presented a greater stimulation response characterized by increased proliferation and proinflammatory cytokine secretion and that compatible to our results that showed low levels of CD4 in patients than controls. The immune system maintains its balance by contrasting activity of different T-cell subpopulations, pathogenic or suppressive, which are regulated by cytokines. Different cytokine profiles of T cells are therefore commonly used to define their functional subsets (Raphael et al., 2015). The CD4+ T-cell subsets are critical for immune homeostasis and host defense, but are also indicated as being a major contributor of pathology in autoimmune and inflammatory diseases, including neurodegenerative diseases (González and Pacheco, 2014). Similar to CD4 Tregs, CD8 Tregs have immunosuppressive functions by secreting various inhibitory cytokines and chemokines. While CD8+ T cells demonstrate cytotoxic effects, CD8 Tregs can effectively block the overreacting immune response to maintain immune homeostasis (Yu et al., 2018). Both CD4+ and CD8+ Tregs display decreased number and/or function in several autoimmune diseases

and have been suggested as immunotherapy targets (Yang et al., 2019). It is hypothesised that a single transient increase in intraocular pressure is enough to induce the expression of heat-shock proteins in the ocular tissue and that the auto-aggressive $CD4^+$ T cells have previously been activated by HSP derived from the gut microbiota (Chen et al., 2018). Recently, using mice deficient in $CD4^+$ αβ T cells, it was reported that $CD4^+$ T cells play a crucial role in propagating retinal ganglioncells (RGCs) degeneration, particularly during the prolonged period of progressive neural damage, in glaucoma (Chen et al., 2018).

Based on FoxP3, our study revealed low levels of FoxP3 marker in patients with glaucoma than healthy. There is no significant different between glaucoma patients with myopia and without myopia and this refer the FoxP3 marker not use in differentiation between glaucoma patients with myopia and without myopia. Study results underline the hypothesis of an immunologic involvement in glaucoma via the cellular immunity, where the Tregs inherit suppressive functions that could be attenuated in glaucoma patients (Bell et al., 2017). Gandolfi et al., (2011) show high levels of FoxP3 in patients than controls and that not compatible to our results. Treg cells play an important anti-inflammatory role in maintaining the peripheral tolerance, controlled by transcription factor Foxp3 and differentiated in response to TGF-β (Guo et al., 2018). Yang et al., (2018) showed decreased ratios of CD4/CD25/FoxP3+ (CD4Treg) and CD8/CD25/FoxP3+ (CD8Treg) in patients than controls. Gandolfi et al., (2017) mentioned the CD4/CD25/FoxP3+ (CD4Treg) lymphocytes can provide biomarkers for POAG. CD4+Foxp3+ regulatory T (Treg) cells have a critical role in the maintenance of immune tolerance and prevention of autoimmunity. Deficiencies in Treg cell development or function result in uncontrolled immune responses accordingly and can lead to inflammatory disorders and autoimmune diseases. Manipulating Treg cells is a promising strategy to treat autoimmunity (Dominguez-Villar and Hafler, 2018). Since depletion of pathogenic $CD4^+$ T cells might lead to suppression of central nervous system autoimmune diseases , it is important to distinguish whether the suppressive effect of αCD4+ interphotoreceptor retinoid-binding protein (IRBP) therapy seen in experimental autoimmune uveitis (EAU)was caused by decreased pathogenic $CD4^+$ effector T cells or by increased $CD4^+Foxp3^+$ Treg cells (Chen et al., 2021).

The conducted study revealed not significant different among all groups (glaucoma patients with myopia, glaucoma patients with myopia, and healthy) according to CD8 and CD4/CD8 ratio markers.

The conducted study revealed there is significant different among all groups (glaucoma patients with myopia, glaucoma patients without myopia, and healthy) relation to TNF_a, TGF_1, and TGF_2 markers. There is high levels of TNF_a, TGF_1, and TGF_2 markers between glaucoma patients with myopia and glaucoma patients without myopia due to hyperinflammatory process in glaucoma patients with myopia. Peng et al., (2020) and Ren et al., (2019) show high levels of TNF_a in patients than controls and that agreed with our results. Tumor necrosis factor-alpha (TNF-a) is a proinflammatory cytokine with pleiotropic effects on various cell types. Enhanced TNF-a expression have been previously reported in AH, trabecular meshwork, retina and optic nerve of clinical glaucomatous specimen, which is consistent with our present finding (Garweg et al., 2017). Currently, biologic agents of anti-TNF-α and anti-IL-6 receptor antibodies are widely used for uveitis treatment with satisfying clinical outcomes (Mesquida et al., 2017). Therefore, there is a possibly important role for these cytokine-related inflammations to play in the pathogenesis of glaucoma , which could provide the therapeutically potential target for glaucoma in the near future (Peng et al 2020). Growing evidence indicates

that TNF-α contributes to the pathogenesis of POAG in several pathways, mostly by induction of apoptosis in RGCs and therefore optic nerve degeneration (Simionescu et al., 2015).

Igarashi et al., (2021) mentioned the Aqueous TGF-β1 and TGF-β levels were significantly higher in patients with glaucoma than controls and that agreed with our results. Various liquid mediators including transforming growth factor-beta (TGF-β), endothelin-1, connective tissue growth factor (CTGF), and several other cytokines have been reported to be upregulated in the aqueous humor (AH) and involved in the increased outflow resistance and Intraocular pressure (IOP) elevation. Past reports have shown that aqueous TGF-β2 is significantly upregulated in POAG than controls (Igarashi et al., 2021). Levels of several mediators such as TGF-βs, vascular endothelial growth factor, CTGF, and monocyte chemoattractant protein-1 are reportedly higher in the AH of POAG, XFG, and neovascular glaucoma patients (Lei et al., 2016).

TGF-β2, a strong fibrotic agent, is upregulated in POAG eyes compared with normal eyes, whereas the levels of aqueous TGF-β2 is somewhat downregulated in SOAG eyes (Inatani et al., 2011). With the hypothesis that some mediators other than TGF-β2 that stimulate Rho/ROCK signaling might exist in SOAG subjects, reporters previously reported that aqueous ATX levels were significantly upregulated in SOAG eyes compared with normal or POAG eyes, and autotaxin was positively correlated with IOP (Honjo et al.,2018). Therefore, we decided to evaluate the possibility that TGF-βs and autotaxin could be factors in differentiating glaucoma subtypes as well as levels of glaucoma severity.

Schlötzer-Schrehardt et al., (2017) reported that TGF-β1 levels were high in exfoliation glaucoma (XFG) and, interestingly, especially high in glaucoma-positive XFG compared with those in exfoliation syndrome (glaucoma-negative) (Garweg et al., 2017). This suggests the involvement of TGF-β1 in the pathogenesis of increased outflow resistance in exfoliation glaucoma (XFG), in concordance with the known role of TGF-β1 in stimulating ECM deposition in many fibrotic disorders. However, as for the high levels of TGF-β1 in SOAG, researchers recently reported that autotoxin and TGF-β1 were upregulated in the aqueous humor (AH) in CMV-positive Posner–Schlossman syndrome (PSS) (glaucoma-positive) (Igarashi et al.,, 2020). In an in vitro study using human TM (hTM) cells, both ATX and TGF-β1 were significantly upregulated during CMV infection, which mimics SOAG in vitro, whereas TGF-β1 was upregulated following ATX upregulation.

Contrary to past studies reporting on the relationship between IOP elevation and TGF-β2 in POAG , there was no significant correlation between IOP and TGF-β2, even when the analysis was restricted to POAG population (Agarwal et al., 2015). Considering the significant IOP elevation in SOAG and XFG patients in clinical practice, this is not surprising. Researchers speculate that TGF-β2 may not be the major factor that induces IOP elevation when considering the overall glaucoma population (Igarashi et al., 2021).

References

- Agarwal, P., Daher, A. M., & Agarwal, R. (2015). Aqueous humor TGF-β2 levels in patients with open-angle glaucoma: A meta-analysis. Molecular vision, 21, 612.
- Bell, K., Holz, A., Ludwig, K., Pfeiffer, N., & Grus, F. H. (2017). Elevated regulatory T cell levels in glaucoma patients in comparison to healthy controls. Current eye research, 42(4), 562-567.

- Chen, H., Cho, K. S., Vu, T. K., Shen, C. H., Kaur, M., Chen, G., & Chen, D. F. (2018). Commensal microflora-induced T cell responses mediate progressive neurodegeneration in glaucoma. Nature communications, 9(1), 1-13.
- Chen, Z., Zhang, T., Kam, H. T., Qiao, D., Jin, W., Zhong, Y., ... & Chen, J. (2021). Induction of antigen-specific Treg cells in treating autoimmune uveitis via bystander suppressive pathways without compromising anti-tumor immunity. EBioMedicine, 70, 103496.
- COMET Group. (2013). Myopia stabilization and associated factors among participants in the Correction of Myopia Evaluation Trial (COMET). Investigative ophthalmology & visual science, 54(13), 7871.
- Czepita, M., Czepita, D., & Safranow, K. (2019). Role of gender in the prevalence of myopia among polish schoolchildren. Journal of ophthalmology, 2019.
- Dominguez-Villar, M., & Hafler, D. A. (2018). Regulatory T cells in autoimmune disease. Nature immunology, 19(7), 665-673.
- Enthoven, C. A., Tideman, J. W. L., Polling, J. R., Yang-Huang, J., Raat, H., & Klaver, C. C. (2020). The impact of computer use on myopia development in childhood: The Generation R study. Preventive medicine, 132, 105988.
- Gandolfi, S. A., Aldigeri, R., Ungaro, N., Tardini, M. G., Paoli, D., & Giacosa, F. (2011). The increased level of circulating and functioning Treg Lymphocytes correlates with visual field rate of progression in Primary Open Angle Glaucoma. Investigative Ophthalmology & Visual Science, 52(14), 4587-4587.
- Gandolfi, S., Ungaro, N., & Varano, L. (2017). Peripheral lymphocytes as a source of biomarkers in POAG. Investigative Ophthalmology & Visual Science, 58(8), 3747-3747.
- Garweg, J. G., Zandi, S., Pfister, I. B., Skowronska, M., & Gerhardt, C. (2017). Comparison of cytokine profiles in the aqueous humor of eyes with pseudoexfoliation syndrome and glaucoma. PloS one, 12(8), e0182571.
- Gomez-Salazar, F., Campos-Romero, A., Gomez-Campaña, H., Cruz-Zamudio, C., Chaidez-Felix, M., Leon-Sicairos, N., ... & Canizalez-Roman, A. (2017). Refractive errors among children, adolescents and adults attending eye clinics in Mexico. International journal of ophthalmology, 10(5), 796.
- González, H., & Pacheco, R. (2014). T-cell-mediated regulation of neuroinflammation involved in neurodegenerative diseases. Journal of neuroinflammation, 11(1), 1-11.
- Guo, C., Wu, N., Niu, X., Wu, Y., Chen, D., & Guo, W. (2018). Comparison of T helper cell patterns in primary open-angle glaucoma and normal-pressure glaucoma. Medical science monitor: international medical journal of experimental and clinical research, 24, 1988.
- Honjo, M., Igarashi, N., Kurano, M., Yatomi, Y., Igarashi, K., Kano, K., ... & Aihara, M. (2018). Autotaxin–lysophosphatidic acid pathway in intraocular pressure regulation and glaucoma subtypes. Investigative ophthalmology & visual science, 59(2), 693-701.
- Hopf, S., Korb, C., Nickels, S., Schulz, A., Münzel, T., Wild, P. S., ... & Schuster, A. K. (2020). Prevalence of myopic maculopathy in the German population: results from the Gutenberg health study. British Journal of Ophthalmology, 104(9), 1254-1259.
- Igarashi, N., Honjo, M., Asaoka, R., Kurano, M., Yatomi, Y., Igarashi, K., ... & Aihara, M. (2021). Aqueous autotaxin and TGF-βs are promising diagnostic biomarkers for distinguishing open-angle glaucoma subtypes. Scientific Reports, 11(1), 1-9.
- Imrie, C., & Tatham, A. J. (2016). Glaucoma: the patient's perspective. British Journal of General Practice, 66(646), e371-e373.

- Inatani, M., Tanihara, H., Katsuta, H., Honjo, M., Kido, N., & Honda, Y. (2011). Transforming growth factor-β 2 levels in aqueous humor of glaucomatous eyes. Graefe's archive for clinical and experimental ophthalmology, 239(2), 109-113.
- Jiang, S., Kametani, M., & Chen, D. F. (2020). Adaptive Immunity: New Aspects of Pathogenesis Underlying Neurodegeneration in Glaucoma and Optic Neuropathy. Frontiers in immunology, 11, 65.
- Koh, V., Tan, C., Tan, P. T., Tan, M., Balla, V., Nah, G., ... & Saw, S. M. (2016). Myopic maculopathy and optic disc changes in highly myopic young Asian eyes and impact on visual acuity. American journal of ophthalmology, 164, 69-79.
- Lei, D., Dong, C., Wu, W. K. K., Dong, A., Li, T., Chan, M. T., ... & Yuan, H. (2016). Lentiviral delivery of small hairpin RNA targeting connective tissue growth factor blocks profibrotic signaling in Tenon's capsule fibroblasts. Investigative ophthalmology & visual science, 57(13), 5171-5180.
- Lin, H. J., Wei, C. C., Chang, C. Y., Chen, T. H., Hsu, Y. A., Hsieh, Y. C., ... & Wan, L. (2016). Role of chronic inflammation in myopia progression: clinical evidence and experimental validation. EBioMedicine, 10, 269-281.
- Mesquida, M., Molins, B., Llorenç, V., de la Maza, M. S., & Adán, A. (2017). Targeting interleukin-6 in autoimmune uveitis. Autoimmunity reviews, 16(10), 1079-1089.
- optometric clinical population. Journal of Optometry.
- Pan, C. W., Dirani, M., Cheng, C. Y., Wong, T. Y., & Saw, S. M. (2015). The age-specific prevalence of myopia in Asia: a meta-analysis. Optometry and vision science, 92(3), 258-266.
- Peng, C., Wu, Y., Ding, X., Chen, D., Zeng, C., Xu, L., & Guo, W. (2020). Characteristic cytokine profiles of aqueous humor in Glaucoma secondary to Sturge-Weber syndrome. Frontiers in immunology, 11, 4.
- Perez, V. L., Pflugfelder, S. C., Zhang, S., Shojaei, A., & Haque, R. (2016). Lifitegrast, a novel integrin antagonist for treatment of dry eye disease. The ocular surface, 14(2), 207-215.
- Pflugfelder, S. C., & de Paiva, C. S. (2017). The pathophysiology of dry eye disease: what we know and future directions for research. Ophthalmology, 124(11), S4-S13.
- Polling, J. R., Klaver, C., & Tideman, J. W. (2021). Myopia progression from wearing first glasses to adult age: the DREAM Study. British Journal of Ophthalmology.
- Raphael, I., Nalawade, S., Eagar, T. N., & Forsthuber, T. G. (2015). T cell subsets and their signature cytokines in autoimmune and inflammatory diseases. Cytokine, 74(1), 5-17.
- Ren, Y., Qi, Y., & Su, X. (2019). Th17 cells in glaucoma patients promote Ig production in IL-17A and IL-21-dependent manner. Clinical and Experimental Pharmacology and Physiology, 46(10), 875-882.
- Ruan, Z., Qian, Z. M., Guo, Y., Zhou, J., Yang, Y., Acharya, B. K., ... & Lin, H. (2019). Ambient fine particulate matter and ozone higher than certain thresholds associated with myopia in the elderly aged 50 years and above. Environmental research, 177, 108581.
- Sawant, D. V., & Vignali, D. A. (2014). Once a Treg, always a Treg?. Immunological reviews, 259(1), 173-191.
- Schaumburg, C. S., Siemasko, K. F., De Paiva, C. S., Wheeler, L. A., Niederkorn, J. Y., Pflugfelder, S. C., & Stern, M. E. (2011). Ocular surface APCs are necessary for autoreactive T cell-mediated experimental autoimmune lacrimal keratoconjunctivitis. The Journal of Immunology, 187(7), 3653-3662.
- Simionescu, R., Cherecheanu, A. P., Voinea, L., & Sfrenț-Cornățeanu, R. (2015). TNF-α Gene polymorphisms and primary open angle glaucoma in Romanian population. Revista Română de Medicină de Laborator Vol, 23(1).

- Wajuihian, S. O., & Mashige, K. P. (2021). Gender and age distribution of refractive errors in an
- Yang, X., Zeng, Q., Göktaş, E., Gopal, K., Al-Aswad, L., Blumberg, D. M., ... & Tezel, G. (2019). T-lymphocyte subset distribution and activity in patients with glaucoma. Investigative ophthalmology & visual science, 60(4), 877-888.
- Yu, Y., Ma, X., Gong, R., Zhu, J., Wei, L., & Yao, J. (2018). Recent advances in CD8+ regulatory T cell research. Oncology letters, 15(6), 8187-8194.