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REVIEW ARTICLE

An association between IL-10 (rs1800896) gene polymorphism, and Open angle glaucoma disease susceptibility in Iraqi patients

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Abstract:

Background: Primary open-angle glaucoma (POAG) is a chronic optic neuropathy that progresses slowly and is characterized by distinctive patterns of optic nerve damage and loss of visual field. It is most common type, a complex and multifaceted disease whose pathogenesis is impacted by both environmental and genetic factors.

Objective: The current study's goal is to determine the relationship between IL10 gene polymorphism, and open angle glaucoma disease susceptibility along with their proposed role in disease pathogenesis.

Methods: Two groups were the subjects of a case-control research: first group included 40 patients who had previously been diagnosed with glaucoma, all cases that were observed in the General Hospital / Diwaniyah / Eye Department and AL Hayat Hospital. From October 2023 to November 2023 under the supervision of an ophthalmologist, and information was collected about each case. From the patient, in addition to the tests that were performed in the center's departments, such as: (eye pressure, age, diabetic, smoking and family history of glaucoma). The second group included 40 healthy volunteers (with no family history of glaucoma). Blood samples were collected by venipuncture from these two groups (five milliliters of venous blood) and were drawn using a disposable syringe under sterile technique. Blood samples is collected in two tubes.

About 2ml of blood has been collected in an EDTA free plain tube and allowed to clot,

then serum was separated by centrifugation 13000 for 5 minutes, serum stored in two parts. Serum has been collected in Eppendorf tube, then stored at -20 to be used for IL-10 and TNF-α ELISA assay as in the study deign figure.

Another 3ml of blood has been collected in EDTA tube for total DNA extraction in order to study Genetic polymorphism for IL-10 SNP (rs1800896), and TNF-α SNP(G308A)

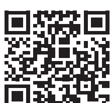
Results: The current investigation found that, when comparing the patient group to the control group, the mean serum IL-10 level was considerably lower., 4.64 ±3.43 versus 6.37 ±2.31, respectively ($p = 0.010$). Additionally, the current investigation found differences in IL10 genotypes between the patient and control groups. Patients had a considerably higher frequency of the genotype GG (42.5 % versus 5%, respectively) than the control group ($p < 0.001$). It is therefore a risk factor with a 14.04 odds ratio. Among patients, Comparing the genotype GA to the control group, it was significantly less common (50.0 % versus 87.5%, respectively; $p < 0.001$). It has an odds ratio of 0.14, making it a protective factor. The frequency of genotype AA in both groups was 7.5%, with no discernible difference ($p = 1.000$).

Conclusion: This study clearly showed that the IL10 (rs1800896) polymorphism are significantly associated with the susceptibility to primary open angle glaucoma in Iraqi patients.

Introduction

Primary open-angle glaucoma (POAG) is a chronic optic neuropathy that progresses slowly and is characterized by distinctive patterns of optic nerve damage and loss of visual field (1). An individual's susceptibility to POAG is a complex disease process that is influenced by a variety of clinical variables, Myopia, age, race, central corneal thickness, intraocular pressure (IOP), and a family history of glaucoma are some of these variables (2)

POAG usually begins slowly, progresses slowly, and causes



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little pain (3). Although it might be asymmetrical, it is typically bilateral, before central vision is impacted in the later stages of the disease, patients may appear to be largely asymptomatic (4). Findings from the examination of the optic nerve, nerve fiber layer, and visual field tests are used to diagnose POAG (5).

For more than 70 million individuals globally, glaucoma is the second most common cause of blindness (6). The incidence of bilateral blindness is approximated at 10%. The frequency of glaucoma in Indonesia is 0.46 percent. This indicates that 4–5% of 1.000 persons have glaucoma (7). Taking into account the irreversible nature of glaucoma, which ranks second in the globe in terms of causes of blindness after cataracts (8). Primary open-angle glaucoma (POAG) impacted 57.5 million persons worldwide in 2015, and 65.5 million in 2020 (8).

According to many researchers, glaucoma is a pressing medical and social problem in all countries, including Morocco (9). Currently, according to the WHO, 105 million people in the world suffer from glaucoma, and according to forecasts, by 2030 this number could double (10).

Known by the name human cytokine synthesis inhibitory factor (CSIF), interleukin-10 (IL-10), Additional investigation shedding light on the critical roles that IL-10 plays in the emergence of disorders related to the eyes (11) Single nucleotide polymorphisms (SNPs),IL-10 serves to shield glaucoma patients from the chronic low-grade inflammatory state that damages eye tissue (12)

Thus, the aim of present study to find out the impact of TNF-α and IL10 gene polymorphism, in open angle glaucoma disease susceptibility along with their proposed role in disease pathogenesis.

2-Methods and Materials

On two groups, case-control research was carried out: first group included 40 patients who had previously been diagnosed with glaucoma, all cases that were observed in the General Hospital / Diwaniyah / Eye Department and AL Hayat Hospital. From October 2023 to November 2023 under the supervision of an ophthalmologist, and information was collected about each case. From the patient, in addition to the tests that were performed in the center's departments, such as: (eye pressure, and other tests). The second group included 40 healthy volunteers (with no family history of glaucoma). These two groups had venipunctures to obtain five milliliters of venous blood, which were then extracted using a disposable syringe while adhering to strict hygiene protocols. Two tubes are used to collect blood samples.

About 3ml of blood has been collected in EDTA tube for total DNA extraction in order to study Genetic polymorphism for IL10 SNP (rs 1800896)

In this study the primer design was carried out according to the complete sequence of IL10(rs1800896) and TNF-α(G308A) obtained from NCBI GenBank data base and Primer 3 plus online which was provided by humanizing genomics Microgen, Korea as following table (1):

Table 1: PCR primers for IL10 SNP (rs1800896).

Primer	Sequence 5'-3		Product Size	Reference
IL10 (rs1800896)	AL allele Reverse primer	CTACTAAGGCTTCTTTGGGAA	550 bp	23
	CL allele Reverse primer	CAGCCCTTCCATTTTACTTTC		
	Common Forward Primer	TACTAAGGCTTCTTTGGGAG		

A little over two milliliters of blood were drawn into an EDTA-free plain tube and allowed to clot. The serum was then separated and kept in two sections using centrifugation at 13000 rpm for five minutes. To be used for the IL10 ELISA assay, serum was collected in an Eppendorf tube and kept at -20 degrees Celsius, as shown in the study design figure.

In the current study the ELISA kit (FAVORGEN / Korea), has been used that uses a single-step, double-antibody sandwich enzyme-linked immunosorbent assay method to measure IL10 in human serum. After spectrophotometric measurement of the color change at 450 nm to determine the IL10 concentration in the samples, the O.D. of the interleukins is compared to a standard curve to calculate the interleukins concentration.

Polymerase Chain Reaction (PCR) technique was performed for detection IL10 SNP (rs1800896) in all patients and in healthy control blood samples as the following steps:

The GoTaq® Green Master Mix Kit (Promega/USA), was used to elaborate the PCR master mix, and the manufacturing company's instructions, as shown in table 2, were followed in the preparation of this master mix.

Table2. PCR Master Mix

Master Mix PCR	The Volume
GoTaq® Green Master Mix	12.5 µl
DNA Template	5 µl
Upstream Primer	2
Downstream Primer	2
Nuclease-free Water	3.5
Total Volume	25 µl

The above-mentioned components of the PCR master mix were then added to standard test tubes together with GoTaq® Green Master Mix, contained all of the elements needed for a PCR reaction, such as Tris-HCl, dNTPs, and Taq DNA polymerase. pH: 9.0, tracking dye, KCl, $MgCl_2$, stabilizer. After that, every PCR tube was moved to the existing vortex centrifuge and spun for five minutes at 3000 rpm.

Afterwards, put in a PCR thermos cycler (Promega/USA).

Tab3.PCR Thermocycler program

Step of PCR	Temperature	Time	No. of Cycles
Saturation	95°C	4 min.	1
Saturation	95°C	30 sec.	35 Cycle
Heating up	54°C	30 sec.	
Adjustment	72°C	25 sec.	
Adjustment	72°C	5 min.	1
Storage	4°C	Hold	-

The PCR amplification products of Hixon gene were analyzed by migration process on agarose gel

electrophoresis as in following steps:

1.Agarose gel (1%) was prepared in using of 1X TBE and dissolving in water bath at 100 °C for 15 minutes and then left to cool 50 °C.
2.Then 0.5µl of previously prepared stock of ethidium bromide stain (1% W/V) was added into agarose gel solution.

3.Agarose gel preparation was poured in tray after fixing the comb in adequate situation and left to be solidified for 15 minutes at room temperature, then the comb was taken away softly from the tray and 10µl of each PCR amplification product for each test samples were added into each comb well and 10µl of (100bp Ladder) in one well as a standard control.

4.The gel tray was stabled in electrophoresis chamber and filled by 1X TBE buffer.

Then electric current was accomplished at 100 volt and 80 Ampere for 60 minutes.

5.PCR amplification products of (560bp) as specific for Hixon gene were visualized by using UV transilluminator.

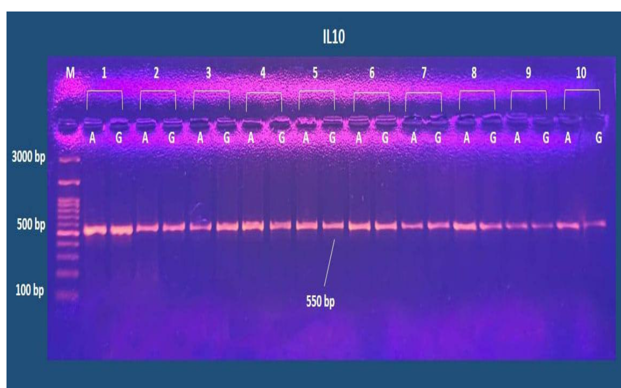

Figure (1): I;10(rs 1800896) the agarose gel electrophoresis picture that revealed the PCR analysis of IL10 SNP gene polymorphisms.

3-Results

Table 4 displays the comparison of serum IL-10 levels between the patient and control groups. The patients' group had a substantially lower mean serum IL-10 than the control group (4.64 ±3.43 versus 6.37 ±2.31, respectively; p = 0.010).

Table 4: Comparison of serum IL-10 between patients' group and control group

Characteristic	group Patients n = 40	group Control n = 40	p
SerumIL-10			
Mean ±SD	4.64 ±3.43	6.37 ±2.31	0.010 I **
Range	1.9 -16.43	3.93 -17.46	

Standard deviation (SD); independent samples t-test (I); significance level (p ≤ 0.001) and significance level (p ≤ 0.01 respectively)

The frequency distribution of patients and control subjects according to IL-10 G/A genotypes is shown in table 5. Overall, genotype GG was reported in 19, genotype GA was seen in 55 and genotype AA was observed in 6. Regarding patients' group, genotype GG was reported in 17, genotype GA was seen in 20 and genotype AA was observed in 3. Regarding control group, genotype GG was reported in 2, genotype GA was seen in 35 and genotype AA was observed in 3.

The distribution in all sample was significantly deviated from expected count based on Hardy Weinberg equilibrium (p <0.001). There was no significant deviation in patients' group (p = 0.377); whereas, significant deviation was seen in control group (p < 0.001).

Table5: The IL-10 G/A genotype frequency distribution in patients and controls

Characteristic	Total n = 80		group Patients n = 40		group Control n = 40	
IL-10 GA	Observed	Expected	Observed	Expected	Observed	Expected
GG	19	27.03	17	18.23	2	9.51
GA	55	38.94	20	17.55	35	19.99
AA	6	14.03	3	4.23	3	10.51
χ^2	13.599		0.780		22.566	
P	< 0.001 ***		0.377 NS		< 0.001 ***	

χ^2: Chi-square test value according to Hardy Weinberg

equilibrium analysis; ***: significant at p ≤ 0.001; NS: not significant

The frequency distribution of IL-10 G/A genotypes in the illness group and control group is contrasted in Table 6. When comparing the patients' genotype GG frequency to that of the control group, it was noticeably higher. at 42.5% versus 5%, respectively (p < 0.001). With an odds ratio of 14.04, it is therefore a risk factor. When comparing the patients' genotype GA to that of the control group, it was found to be substantially less common (50.0 % versus 87.5%, respectively; p < 0.001). With an odds ratio of 0.14, it is therefore a protective factor. Between the two groups, the frequency of genotype AA was 7.5% versus 7.5%, respectively, with no appreciable difference observed (p = 1.000).

Table6: Comparison the frequency distribution of IL10 G/A genotype in patients and control groups

IL-10 genotypes	group Patients n = 40	group Control n = 40	p	OR	95 % CI
GG, n (%)	17 (42.5 %)	2 (5.0 %)	< 0.001 C ***	14.04	2.97 - 66.43
GA, n (%)	20 (50.0 %)	35 (87.5 %)	< 0.001 C ***	0.14	0.05 -0.44
AA, n (%)	3 (7.5 %)	3 (7.5 %)	1.000 F NS	1.00	0.06 - 16.56

The terms OR, CI, C, F, and NS denote odds ratio, confidence interval, chi-square test, and Fischer exact test, respectively. *** indicates significant at p < 0.001.

4- Discussion

Human cytokine synthesis inhibitory factor (CSIF), also known as interleukin-10 (IL-10), has been linked to the development of eye-related illnesses (13)

IL-10 serves to shield glaucoma patients from the chronic low-grade inflammatory state that damages eye tissue. It is a crucial regulator of the systemic anti-inflammatory responses. According to Abos et al. (14), IL-10 improves human B cell survival, proliferation, differentiation, and isotype switching. Similar to what was found in the current investigation, Irkec et al (15), study results indicated low levels of IL-10 production in glaucomatous people. The varied age profiles, different disease durations and activities, and different treatment(s) obtained by the patients could all account for this variance in the inflammatory biomarkers' results (16).

Dong et al found that there was a strong negative connection between the severity of DR and the aqueous humor's IL-10 levels (17).

Interleukin 10 (IL10), is a type of cytokine that controls inflammation and immunological responses. Many disorders, including glaucoma, have been linked to genetic variants in the IL10 gene.

Cytokine IL10 plays a role in controlling inflammation and immunological reactions. Dysregulated immunological responses brought on by genetic variants in IL10, such the GG genotype, may aid in the onset or advancement of POAG (18). A glaucoma-causing optic nerve injury could result from increased inflammation or immunological activation in the eye (19).

In the context of POAG, dysregulation of the anti-inflammatory response can have detrimental effects on ocular tissues, particularly the optic nerve head. One of the risk factors for POAG is elevated intraocular pressure (19), and it can induce mechanical stress and trigger inflammatory processes in the optic nerve head. IL-10, when functioning properly, helps to mitigate this inflammation and protect against optic nerve damage. However, genetic variants like the GG genotype may compromise the ability of IL-10 to counteract inflammation effectively, aggravating optic nerve damage and aiding in the onset and advancement of POAG (20).

GG genotype may be linked to changed IL10 expression or activity levels, according to the present study the genotype GG was significantly more frequent in patients in comparison with control group.

Associations between the GG genotype and POAG suggest that this genetic variant may contribute to the pathogenesis of the disease, possibly through its effects on inflammation and immune regulation (20).

Previous studies in the Turkish community showed that at the IL-10 −1082 (A/G) polymorphism site. The frequency of the GG genotype varied significantly between the sick population and the control group. This genotype was more common in the control group.

In a study conducted in Iran by Fakhraie et al in 2020, which agreed with the results we reached (GG and GA increase in patent than control) (21).

Kapuganti et al (22), disagree with present study, they found that there is a substantial correlation between the AA genotype of the -1082A/G SNP with the likelihood of developing PEX or PEXG and POAG diseases . These variations could be explained

by the patients' clinical heterogeneity, sample size, diversity in ethnicity, and poorly defined controls.

Conclusion

Current study provides evidence that the IL-10 (Rs 1800896) variant related to susceptibility in Iraqi patient with POAG, the genotype GG was significantly more frequent in patients in comparison with control group ($p < 0.001$). Thus, it considers a risk factor with an odds ratio of 14.04. The genotype GA was significantly less frequent in patients in comparison with control group ($p < 0.001$). Thus, it is a protective factor with an odds ratio of 0.14. There was no significant difference in the frequency of genotype AA between both groups ($p = 1.000$).

REFERENCES

1. Sharif, N. A. (2022). Neuropathology and Therapeutics Addressing Glaucoma, a Prevalent Retina-Optic Nerve-Brain Disease that Causes Eyesight Impairment and Blindness. OBM Neurobiology, 6(1), 1-51.
2. Ciociola, E. C., Anderson, A., Jiang, H., Funk, I., Lin, F. C., Mwanza, J. C., ... & Fleischman, D. (2023). Decision Factors for Glaucoma Suspects and Ocular Hypertensive Treatment at an Academic Center. Journal of Current Glaucoma Practice, 17(3), 157.
3. Gillmann, K., & Mansouri, K. (Eds.). (2023). The Science of Glaucoma Management: From Translational Research to Next-Generation Clinical Practice. Elsevier.
4. Dietze, J., Blair, K., Havens, S. J., & Adams, M. (2021). Glaucoma (Nursing).
5. Kastner, A., & King, A. J. (2020). Advanced glaucoma at diagnosis: current perspectives. Eye, 34(1), 116-128.
6. Firan, A. M., Istrate, S., Iancu, R., Tudosescu, R., Ciuluvică, R., & Voinea, L. (2020). Visual evoked potential in the early diagnosis of glaucoma. Literature review. Romanian Journal of Ophthalmology, 64(1), 15.
7. Usgaonkar, U. P., Naik, R., & Shetty, A. (2023). The economic burden of glaucoma on patients. Indian Journal of Ophthalmology, 71(2), 560.
8. Angriani, N., Akib, M. N., Mulyadi, F. E., Irmandha, S., Hadi, S., & Syamsu, R. F. (2022). Glaucoma risk factors. Green Medical Journal, 4(1), 1-8.
9. Sari, P. M., & Yunanda, R. (2022). Characteristics of Primary Glaucoma in Glaucoma Clinic of Dr. Mohammad Hoesin General Hospital, Palembang. Sriwijaya Journal of Ophthalmology, 5(1), 155-159.
10. Wang, H. W., Sun, P., Chen, Y., Jiang, L. P., Wu, H. P., Zhang, W., & Gao, F. (2018). Research progress on human genes involved in the pathogenesis of glaucoma. Molecular medicine reports, 18(1), 656-674.
11. Tan, W., Zou, J. L., Yoshida, S., Jiang, B., & Zhou, Y. D. (2020). Increased vitreal levels of interleukin-10 in diabetic retinopathy: a Meta-analysis. International Journal of Ophthalmology, 13(9), 1477.
12. Tezel, G. (2022). Molecular regulation of neuroinflammation in glaucoma: Current knowledge and the ongoing search for new treatment targets. Progress in retinal and eye research, 87, 100998.
13. de Almeida, I. D. M., de Paiva, P. L., Santos, K. D. F., Reis, A. A. D. S., & Santos, R. D. S. (2023). IL10 Gene and Neurodegenerative Sclerosis: A Systematic Review and Meta-Analysis. Journal of Molecular Pathology, 4(4), 213-224.
14. Balderrama-Gutierrez, G., Milovic, A., Cook, V. J., Islam, M. N., Zhang, Y., Kiaris, H., ... & Barbour, A. G. (2020). The infection-tolerant mammalian reservoir of Lyme disease and other zoonoses broadly counters the inflammatory effects of endotoxin. bioRxiv, 2020-12.
15. Yucel Gencoglu, A., Irkec, M., Kocabeyoglu, S., Dikmen, Z. G., Karakaya, J., & Konstas, A. G. (2022). Plasma levels of sirtuin and adiponectin in patients with primary open-angle glaucoma, exfoliative glaucoma, and healthy controls. European Journal of Ophthalmology, 32(5), 2893-2898.
16. Azemi, A. K., Nordin, M. L., Hambali, K. A., Noralidin, N. A., Mokhtar, S. S., & Rasool, A. H. G. (2022). Phytochemical contents and pharmacological potential of Parkia speciosa Hassk. for Diabetic Vasculopathy: A Review. Antioxidants, 11(2), 431.
17. Dong, N., Xu, B., Wang, B., & Chu, L. (2013). Study of 27 aqueous humor cytokines in patients with type 2 diabetes with or without retinopathy. Molecular vision, 19, 1734.
18. Kumaramanickavel, G., Jadhav, S., Ramsait, S. P., & Davey, P. G. (2022). Molecular Genomics of Glaucoma: An Update. In Glaucoma-Recent Advances and New Perspectives. IntechOpen.
19. Duarte, J. N. (2021). Neuroinflammatory mechanisms of mitochondrial dysfunction and neurodegeneration in glaucoma. Journal of Ophthalmology, 2021, 1-18.
20. Atanasovska Velkovska, M., Goričar, K., Blagus, T., Dolžan, V., & Cvenkel, B. (2021). Association of genetic polymorphisms in oxidative stress and inflammation pathways with glaucoma risk and phenotype. Journal of clinical medicine, 10(5), 1148.
21. Fakhraie, G., Parvini, F., Ghanavi, J., Saif, S., & Farnia, P. (2020). Association of IL-10 gene promoter polymorphisms with susceptibility to pseudoexfoliation syndrome, pseudoexfoliative and primary open-angle glaucoma. BMC Medical Genetics, 21, 1-8.

22. Kapuganti, R. S., & Alone, D. P. (2023). Current understanding of genetics and epigenetics in pseudoexfoliation syndrome and glaucoma. Molecular Aspects of Medicine, 94, 101214.

23. Braga, M., Lara-Armi, F. F., Neves, J. S. F., Rocha-Loures, M. A., de Souza Terron-Monich, M., Bahls-Pinto, L. D., ... & Visentainer, J. E. L. (2022). Influence of IL10 (rs1800896) Polymorphism and TNF-a, IL-10, IL-17A, and IL-17F Serum Levels in Ankylosing Spondylitis. Recent Advances in Potential Biomarkers for Rheumatic Diseases and in Cell-based Therapies in the Management of Inflammatory Rheumatic Diseases.