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Serum IL-6/ IL-10 Ratio As A Biomarker for Primary Open Angle Glaucoma Assessment

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ABSTRACT

Background: Substantial studies have been conducted on the function of interleukin-6 (IL-6) in various eye problems. While the versatile nature of IL-6 is well-documented, its primary role is to induce inflammation through its cytokine activity.

Objectives: The current work aimed to use serum IL-6 and IL-6/IL-10 ratio as potential biomarkers for primary open angle glaucoma (POAG) assessment.

Patients and Methods: 40 individuals were enrolled in this case-control study at Benha University hospitals' Ophthalmology, and Clinical and Chemical Pathology Departments. The participants were divided into two distinct groups: the case group consisted of twenty patients diagnosed with POAG and the control group included twenty healthy individuals who did not have glaucoma or any other ocular disorders. The cytokines levels of each participant were assessed using ELISA kits for serum IL-6 and IL-10.

Results: POAG and control groups showed no significant difference in age and sex. Regarding serum cytokines levels as diagnostic markers, POAG patients had a higher level of IL-6 and a lower level of IL-10 than control subjects. The IL-6 /IL-10 ratio displayed significantly higher values in POAG cases than controls. Regarding serum cytokines levels as prognostic markers, a significantly higher IL-6 level, and IL-6/IL-10 ratio were observed in the severe stage relative to the mild-moderate stages with no significant difference in the serum IL-10 levels.

Conclusions: It could be concluded that the serum IL-6 and IL-6/IL-10 ratio is potentially useful as biomarkers for diagnosis and evaluation of the severity of POAG. Consistently monitoring serum concentrations of IL-6 and IL-10 may be used as supplementary laboratory methods in the POAG assessment.

Keywords: Cytokines; Serum Il-6/Il-10 Ratio; Biomarker; Primary Open Angle Glaucoma

INTRODUCTION

Glaucoma is distinguished by the gradual deterioration of optic nerve head (ONH) retinal ganglion cells (RGCs) and axons. Significantly, intraocular pressure (IOP) contributes to the disease's progression.[1] Open-angle glaucoma (OAG) and angle-closure glaucoma are both the two broad categories of glaucoma which can be primary or secondary diseases. Although OAG accounts for more than 80% of cases diagnosed in the United States, angle-closure glaucoma contributes to a disproportionately high proportion of cases characterized by severe visual impairment.[2]

OAG is an irreversible, chronic, and progressive optic neuropathy that is brought on by a confluence of numerous contributing factors. Characteristics of this condition include an open anterior chamber, typical changes of ONH, and corresponding visual field (VF) loss. With time, it ends with blindness. The disease is usually bilateral but asymmetrical and IOP is a main modifiable threat element [3]. POAG is the most common type. Patients diagnosed with POAG have been seen to demonstrate dysregulation of systemic inflammatory cytokines, including TNF- α, IL-1 , IL-4, IL-6 and IL-12 [4]. These results support the notion that aberrant systemic immunological conditions have a significant effect on the neurodegenerative process of glaucoma patients [5].

Considerable research has been devoted to examining the involvement of IL-6 in a variety of ocular disorders. Despite the well-known versatility of IL-6, its principal function remains as an inflammatory cytokine [6,7]. Glaucoma-associated fibrosis of the human trabecular meshwork (HTM) is predominantly governed by the interplay between transforming growth factor beta (TGF- β) and IL-6. Furthermore, IL-6 has the potential to function as an indicator of impaired axonal transport in experimental glaucoma. Most research has been devoted to IL-6 production in the eye, although it remains unknown how systemic IL-6 contributes to the pathogenic mechanism of glaucoma. Anti-inflammatory response as opposed to IL-6 is where IL-10 truly excels [8]. IL-10 promoter polymorphisms have been linked with POAG susceptibility in numerous ethnic group investigations. IL-10 production has been observed to decrease in glaucomatous individuals carrying the IL-10 haplotypes −1082, −819, or −592. In summary, these results provide support for the idea that the aberrant systemic immune environment of individuals with glaucoma shows a substantial part in the formation and progress of neurodegeneration [8, 9].

Glaucoma frequently manifests with a gradual onset, and the alterations in both structure and function may remain imperceptible for several years. Despite the potential for visual abnormalities to be recognized prior

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to significant RGCs loss, the VF test or perimetry remains the mainstay of assessing visual function and progression of glaucoma [10].

This study aimed to use serum IL-6 and IL-6/IL-10 ratio as potential biomarkers for POAG assessment.

PATIENTS AND METHODS:

This case-control trail study included a total of 20 patients diagnosed with POAG and 20 age matched controls, attending at Departments of Ophthalmology, and Clinical and Chemical Pathology, Benha University Hospitals. This study was conducted between June 2022 and July 2023.

Inclusion criteria: Absence of other causes for optic neuropathy rather than glaucoma. The following signs were identified: open anterior chamber angles, cupping of the optic nerve and retinal nerve fiber layer thinning, characteristic glaucomatous changes in the VF testing, IOP over 22 mmHg, and the lack of signs of secondary glaucoma [11].

Exclusion criteria: Cases of non-POAG glaucoma, other eye disorders (including retinal diseases, periocular inflammation, and ocular diseases caused by immunologic disorders), systemic diseases (including kidney, diabetes mellitus, cardiovascular diseases or infectious) inflammation (defined as any inflammation occurring within the previous six months), and a history of systemic drug therapy (as non-steroidal anti-inflammatory drugs, chemotherapeutic agents, and corticosteroids).

Cytokine measurement:

Blood samples were taken first in the morning for all participants. The serum was subsequently separated and preserved at -20°C until analysis. The cytokine profiles were quantified in accordance with the manufacturer's instructions using ELISA kits for human IL-6 and IL-10, both of which were produced by the Bioassay Technology Laboratory in Shanghai, China.

Ethical considerations: The study was done after being accepted by the Research Ethics Committee, Benha University. Written informed consent of all the participants was obtained after being informed about the study's requirements, objectives, and potential risks. The consent form explicitly outlined their agreement to participate in the study and for the publication of data, ensuring the protection of their confidentiality and privacy. This work has been carried out following The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

The collected data was analyzed using Statistical Package for the Social Sciences (IBM SPSS Statistics for Windows, Version 25.0, IBM Corp., 2017). In descriptive statistics, the mean and standard deviation (± SD) are presented for numerical data that adheres to a normal distribution. When numerical data deviates from a normal distribution, the measures of median and range are applied. Frequency and percentage were employed to present non-numerical statistics. Statistical significance was established at a level of confidence of 95 percent using a p-value below 0.05.

RESULTS

Case and control groups showed no statistically significant difference regarding age and sex. (**Table 1).**

Table 1: Demographic data of the studied groups

		Case group (n=20)	Control group (n=20)	P-value
Age (Years)	Mean ±SD	53.82± 12.92	45.32± 11.59	0.061
	Range	25 - 73	27 – 68	
Sex	Male	12 (63.64%)	10 (50%)	0.087
	Female	8 (36.36%)	10 (50%)	

*Significant as P value ≤0.05.

In terms of serum cytokine concentrations, a substantial elevation in IL-6 and the IL-6/IL-10 ratio was observed in the cases compared to to the control group (P <0.001). A significantly reduced concentration of IL-10 was observed in the cases compared to the control group (P <0.001). (**Table 2).**

Table 2: Interleukins and ratio of the studied groups

		Case group (n=20)	Control group (n=20)	P-value
Interleukin-6 (pg/ml)	Mean ±SD	1814.36± 412.28	969.08± 207.11	<0.001*
Interleukin-10 (pg/ml)	Mean ±SD	157.91± 40.36	198.69± 36.31	<0.001*
IL-6/IL-10 Ratio	Mean ±SD	9.78± 1.15	5.3± 1.03	<0.001*

*Significant as P value≤0.05

To further investigate their role in severity evaluation, a comparison was conducted between the mild-moderate group (MD of ≤ -12 dB) and the severe group (MD of >-12 dB) to throw light on their statistical relevance as markers of POAG progression. IL-10 levels did not differ significantly between the early-moderate and severe groups (p=0.061). Nevertheless, the severe group exhibited notably elevated levels of IL-6 and the IL-6/IL-10 ratio (p=0.001, p=0.009). Consequently, there was no statistically significant change observed in serum IL-10 levels. Nevertheless,

severe cases demonstrated heightened concentrations of serum IL-6 and an IL-6/IL-10 ratio. (**Table 3).**

Table 3: Interleukins and ratio of severe and mild-moderate groups.

		Severe (n=6)	Mild to moderate (n=14)	P-value
Interleukin-6 (pg/ml)	Mean ±SD	2341.7± 62.5	1267.95± 216.16	0.001*
Interleukin-10 (pg/ml)	Mean ±SD	222.1± 8.4	197.77± 25.37	0.061
IL-6/IL-10 Ratio	Mean ±SD	10.8±2	9.91±1.76	0.009*

*Significant as P value≤0.05

DISCUSSION

A collection of optic neuropathies, glaucoma causes irreversible visual impairment by progressively destroying RGCs. Caused primarily by RGC apoptosis, it is the most prevalent form of permanent blindness worldwide [12]. Many patients with this condition do not receive a diagnosis until they have already experienced significant vision loss due to the lack of obvious symptoms in the early stages of the disease and the difficulty in making a clinical diagnosis. This emphasizes the need for effective glaucoma screening tools for early diagnosis. Timely intervention is also necessary to avoid blindness. [13].

While a high IOP is a useful indicator of glaucoma, it is not conclusive. Although some patients with POAG do not experience elevated IOP, not all patients presenting with a raised intraocular pressure (IOP) will develop glaucoma. This variation underscores complexity of the ailment and underscores the necessity for supplementary biomarkers to support the diagnosis, treatment, and overall comprehension of POAG [14]. Inflammation has recently attracted the attention of scientists as a possible glaucoma etiological theory. The hypothesis that POAG contributes to the pathogenesis of chronic low-grade inflammation is gaining support. An important pro-inflammatory cytokine in this process is IL-6.[15].

There are numerous physiological and pathological processes that rely on IL-6, a multifunctional cytokine, including the regulation of immune responses and tissue repair. The pro-inflammatory cytokine role of IL-6 is still its most prominent one, despite its well-known flexibility. The fact that POAG patients' aqueous humor (AH) and serum IL-6 levels are higher than normal suggests that this biomarker could be valuable [16]. In comparison to IL-6, IL-10's anti-inflammatory properties have received more attention. Inhibition of pro-inflammatory cytokine production and understanding of immune response modulation suggests it may have a protective effect on RGCs [17].

Since IL-6 and IL-10 play different roles in inflammation, the IL-6/IL-10 ratio has now come to light as a potential glaucoma biomarker. In the search process for reliable biomarkers to identify and monitor the progression of POAG, the intricate interplay among cytokines like IL-6 and IL-10 opens new avenues for investigation [5].

Consequently, the aim of this study's activity was to investigate the potential of serum IL-6 levels and the IL-6/IL-10 ratio as prognostic indicators for POAG. The results of this research indicated that the levels of IL-6 and the IL-6/IL-10 ratio in the patients were significantly higher than those in the control group ($p < 0.001$ for IL-6 and $p < 0.001$ for IL-6/IL-10 ratio, respectively).

In line with our results, **Ulhaq *et al.*** [5] observed that the levels of IL-6 were noticeably greater in POAG cases when contrasted with control subjects ($p < 0.0001$). The POAG cases had noticeably greater IL-6/IL-10 ratio values ($p < 0.0001$) in comparison to the control group.

Other studies conducted on the AH, **Ghanem *et al.*** [18] study revealed that the POAG group's IL-6 concentration was significantly higher than the control group's (P=0.005). Also, **Yanhong *et al.*** [19] study found that the amounts of IL-6 in both AH and plasma were considerably higher in those with POAG compared to those with age-related cataracts ($P<0.01$ and $P<0.05$, respectively).

As opposed to our results, **Sorkhabi *et al.*** [20] study found non statistically significant difference between the control and POAG IL-6 concentrations in the serum (p=0.112) or the AH (p=0.14).

Besides, in **Takai *et al.*** [21] study, In comparison to the control group of eyes with cataracts, the IL-6 level was considerably lower in the POAG group (0.23-fold) (p= 0.0123). **Borkenstein *et al.*** [22] were shown to be associated with reduced IL-6 levels in POAG eyes. People with POAG had much lower mean AH levels of IL-6 than those in the control group (p=0.002).

The levels of IL-10 were considerably lower in the case group when contrasted with the control group, according to our findings. The significance level is less than 0.001.

Furthermore, **Kokubun *et al.*** [23] demonstrated that the POAG group had a substantially lower AH IL-10 rate than the cataract group and the neovascular glaucoma group (P = 0.0002 and 0.0044, respectively).

Contrary to our results, **Ulhaq *et al.*** [5] found no significant difference (p = 0.083) in the mean IL-10 levels between the control group and the patients.

For further examination, the severe group (n=12) and the mild to moderate groups (n=32) were compared once more. IL-6 and the IL-6/IL-10 ratio were both considerably higher in the severe cases group than in the mild to moderate cases group (p = 0.001 and p = 0.009, respectively), although serum IL-10 levels were not significantly different (p value=0.061).

Huang *et al.* [24] divided 32 POAG patients according to their MD, with more than -12 dB being

considered severe glaucomatous neuropathy (P=0.0084) showing higher IL-6 concentrations and less than -12 dB as mild glaucomatous neuropathy (P=0.0302) showing lower IL-6 concentrations.

The study of **Irkec *et al.*** [25] reached the conclusion that IL-10 polymorphism and glaucoma severity were not correlated (p=0.081).

CONCLUSIONS

It could be concluded that the serum IL-6 and IL-6/IL-10 ratio is potentially useful as biomarkers for diagnosis and evaluation of the severity of POAG. Consistently monitoring serum concentrations of IL-6 and IL-10 may be used as supplementary laboratory methods in the POAG assessment.

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