Advance Sustainable Science, Engineering and Technology (ASSET)
Vol. 8, No.3, July 2026, pp. 02603024-01 ~ 02603024-08
ISSN: 2715-4211 DOI: https://doi.org/10.26877/asset.v8i3.2383

Exploring Serum Bilirubin as a Potential Biomarker for Primary Open-Angle Glaucoma

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Abstract. Total bilirubin (TBIL) functions as a natural antioxidant, and its levels tend to decrease with increasing oxidative stress, including in glaucoma tissue. This study aims to explore the relationship between total serum bilirubin levels and the severity of primary open-angle glaucoma (POAG). A total of 25 POAG patients and 25 control subjects further divided into aged >60 years and ≤60 years from the Glaucoma Clinic of the Universitas Sumatera Utara Hospital and its affiliated hospitals were included, consisting of 4 patients with moderate disease and 21 with advanced disease. Serum TBIL levels were measured and analyzed using the Mann-Whitney test. Results showed an average TBIL of 0.23 mg/dL in moderate cases and 0.37 mg/dL in advanced cases, with no significant relationship between TBIL and POAG severity (p=0.131). These findings indicate that total serum bilirubin levels are not related to POAG severity and are likely not a reliable clinical biomarker for glaucoma.

Keywords: serum bilirubin, oxidative stress, biomarkers, severity of POAG.

(Received 2025-07-16, Revised 2025-09-17, Accepted 2025-10-10, Available Online by 2026-06-11)

1. Introduction

The progressive deterioration of retinal ganglion cells and their axons is a hallmark of glaucoma, making it a leading cause of permanent blindness worldwide [1], [2]. This disease is multifactorial, where a combination of genetic factors, high intraocular pressure, vascular dysfunction, and systemic factors such as diabetes can influence its development and severity [3], [4]. The most prevalent form of

glaucoma is primary open-angle glaucoma (POAG), which is marked by an open anterior chamber angle and the gradual, irreversible deterioration of retinal ganglion cells and the visual field [5], [6], [7].

One of the main mechanisms in the pathophysiology of POAG is oxidative stress. Increased free radicals and decreased antioxidant capacity can damage retinal ganglion cells and optic tissue, contributing to optic nerve degeneration. Therefore, molecules associated with oxidative stress are potential candidates as biomarkers for detecting and predicting the progression of glaucoma [8], [9].

Total bilirubin (TBIL) is an endogenous antioxidant that has been shown to combat oxidative stress [10], [11]. Several studies have shown that higher TBIL levels have a protective effect against oxidative damage, while low levels may reflect increased oxidative stress. Research by Mingxi Shao et al., [12] indicates that bilirubin may serve as an indicator of oxidative and cytotoxic stress that influences physiological and pathological mechanisms in glaucoma. The findings also indicate a gender-specific relationship between TBIL and POAG. Similarly, Dong et al. [13] found a correlation between elevated TBIL levels and fundus arteriosclerosis in men, but not in women. This aligns with prior studies that have established a tendency for men to have higher blood bilirubin levels compared to women.

However, the relationship between serum TBIL levels and the severity or incidence of POAG is still limited, especially in the Indonesian population, as most of the evidence comes from foreign studies. The novelty of this study lies in its focus on the local population, its analysis of the relationship between TBIL and the severity of POAG, and its consideration of gender as a moderating factor, so that the results can provide clinical insights for early detection and management of the disease.

The results of this study are expected to provide new insights into early detection, disease progression prediction, and more targeted POAG management strategies. These findings are also expected to strengthen understanding of the role of endogenous antioxidants in POAG pathogenesis, provide a scientific basis for the development of new clinical indicators, and assist in identifying patients at risk of rapid progression, thereby enabling earlier treatment intervention and improved personalization of POAG management.

2. **Methods**

To determine the connection between total serum bilirubin levels and POAG severity, this research utilized an analytical observational design with a cross-sectional approach.

2.1 Place and Time of Research

Data were collected from POAG patients and control groups at the Universitas Sumatera Utara Hospital and its affiliated hospitals. This study was conducted from November 2023 to March 2024 after obtaining ethical approval from the Universitas Sumatera Utara Faculty of Medicine Health Research Ethics Committee.

2.2 Population and Research Sample

The case population consists of all individuals who have been diagnosed with POAG, while the accessible population includes POAG patients who are treated at the Ophthalmology Polyclinic of the Glaucoma Division of Universitas Sumatera Utara Hospital and its affiliated hospitals. Participants were eligible for inclusion if they were 18 or older, had a POAG diagnosis, and provided written consent. Those with a history of chronic liver disease, hemolytic disease, bilirubin metabolism disorders, or those using drugs that impact bilirubin levels were not included.

The control group consisted of individuals with clinically normal eyes, normal intraocular pressure, and no history of glaucoma, liver disease, or bilirubin metabolism disorders. Samples were taken purposively from the accessible population until the target number was reached.

2.3 Data Collection Methods

1. Submitted a research application letter at the Department of Ophthalmology of Faculty of Medicine of Universitas Sumatera Utara / Universitas Sumatera Utara Hospital and Medan Baru Special Eye

Hospital.

2. Apply for an "ethical clearance" research permit from the Health Research Ethics Committee of Faculty of Medicine Universitas Sumatera Utara to research sites at Universitas Sumatera Utara Hospital and affiliated hospitals.
3. Provide an explanation to the research subjects and the patient's family about the stages of examination in this study.
4. Conducting research data collection at the ophthalmology polyclinic of the Glaucoma division of Universitas Sumatera Utara Hospital and Network Hospital
5. Conducting clinical examinations to confirm POAG diagnosis, including intraocular pressure, visual field, and anterior chamber angle evaluations.
6. Taking venous blood samples for total serum bilirubin measurement. Analysis was performed using the COBAS Integra 400 Plus device, with standard calibration and laboratory reference values of 0.2–1.2 mg/dL. Each sample was analyzed in duplicate to ensure reproducibility of results.

2.4 Research Flow

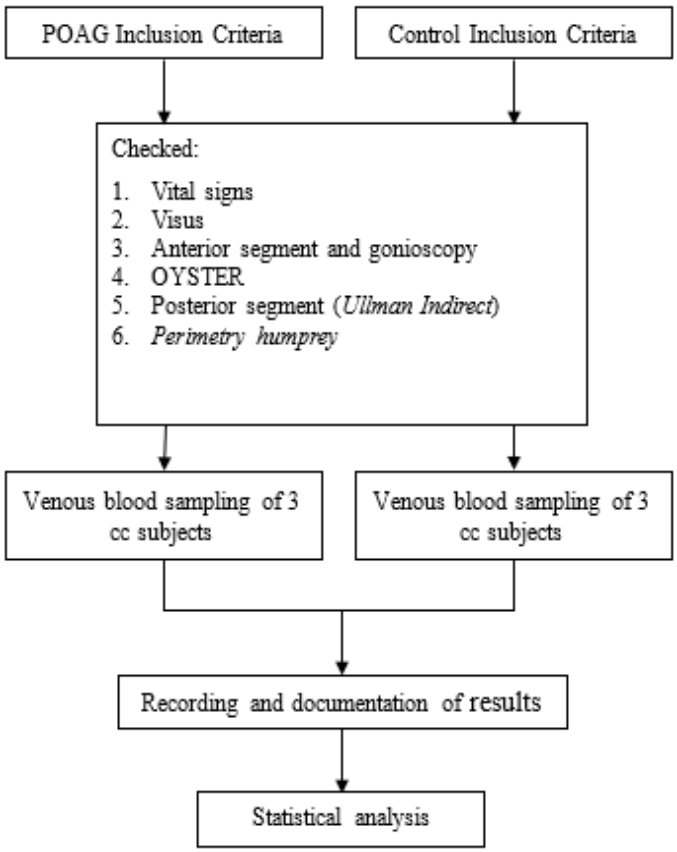

Figure 1. Research Flow

2.5 Data Analysis

Descriptive and analytical methods were used to analyze the data. Categorical variables are shown as frequency distributions, and numerical variables are presented as either the mean and standard deviation for normally distributed data or the median (with a range from minimum to maximum) for non-normally distributed data, as determined by a Shapiro-Wilk test.

To test the relationship between TBIL and POAG severity, the Independent T-test was used if the data

were normally distributed, or the Mann-Whitney test for non-normal data; the choice of test was adjusted to the data distribution so that statistical assumptions were met. Correlation analysis was performed using the Pearson test for normal data and the Spearman test for non-normal data. Statistical significance was set at $p < 0.05$.

3. Results and Discussion

3.1 Demographic Characteristics of Subjects

This study involved 25 POAG patients from the Glaucoma Division of the Ophthalmology Polyclinic at the Universitas Sumatera Utara Hospital and its affiliated hospitals, as well as 25 control subjects with normal eyes from the same institution. Demographic analysis showed that female subjects dominated both groups (76% POAG and 60% control), but this difference was not significant (p=0.255). Subjects aged >60 years were also more numerous in both groups (76% POAG and 80% controls), with no significant difference (p=0.733) (Table 1).

Table 1. Demographic Characteristics of Subjects

Characteristics	POAG (n=25)	Control (n=25)	p
Gender, n (%)			
Male	6 (24%)	10 (40%)	0,255*
Female	19 (76%)	15 (60%)	
Age, n (%)			
>60 year	19 (76%)	20 (80%)	0,733*
≤60 year	6 (24%)	5 (20%)	

*Chi-square test

3.2 Total Bilirubin Levels in POAG and Controls

The mean total bilirubin (TBIL) level in the POAG group was 0.36 mg/dL, slightly lower than the control group at 0.40 mg/dL, but this difference was not significant (p=0.503, Mann-Whitney) (Table 2).

Table 2. Total Bilirubin Levels in POAG and Controls

Variabel	POAG (n=25)	Control (n=25)	p
Average TBIL (mg/dL)	0,36 ± 0,15	0,40 ± 0,18	0,503*
Median (Min–Max)	0,34 (0,13–0,67)	0,38 (0,14–0,97)	

*Mann-Whitney test

3.3 Degree of POAG and Family History

A total of 21 subjects (84%) showed advanced POAG and 4 subjects (16%) had moderate POAG. In terms of family history, 14 subjects (56%) had family members with POAG (Table 3).

Table 3. Degree of POAG and Family History

Variabel	n (%)
POAG Degree	
Moderate	4 (16%)
Advanced	21 (84%)
Family History of POAG	
Yes	14 (56%)
No	11 (44%)

3.4 Relationship between Total Bilirubin Levels and POAG Severity (Subgroups)

Analysis of the relationship between TBIL and POAG severity showed that the mean TBIL in moderate POAG was 0.25 mg/dL, while in advanced POAG it was 0.38 mg/dL (p=0.110, Mann-Whitney). In

female subjects, the mean TBIL was 0.25 mg/dL in moderate POAG and 0.33 mg/dL in advanced POAG (p=0.150). Subjects >60 years of age had a mean TBIL of 0.23 mg/dL in moderate POAG and 0.37 mg/dL in advanced POAG (p=0.131). Analysis based on family history showed a mean TBIL of 0.18 mg/dL in moderate POAG and 0.37 mg/dL in advanced POAG in subjects with a family history (p=0.097), while in subjects without a family history, the mean TBIL was 0.32 mg/dL in moderate POAG and 0.39 mg/dL in advanced POAG (p=0.545) (Table 4). All male subjects were in advanced POAG and therefore could not be analyzed statistically.

Table 4. Relationship between Total Bilirubin Levels and POAG Severity (Subgroups)

Subgroup	Moderate POAG	Advanced POAG	p
All subjects	0,25 ± 0,08	0,38 ± 0,15	0,110*
Female	0,25 ± 0,08	0,33 ± 0,10	0,150T
Age >60 years	0,23 ± 0,09	0,37 ± 0,15	0,131*
Family history: Yes	0,18	0,37 ± 0,14	0,097T
Family history: No	0,32 ± 0,04	0,39 ± 0,17	0,545T

*Mann-Whitney test, T Independent

Overall, there was no significant relationship between total serum bilirubin levels and the severity of POAG, regardless of gender, age, or family history.

Discussion

Primary open-angle glaucoma (POAG) is a chronic and progressive optic neuropathy characterized by optic disc cupping and atrophy, visual field loss, and open anterior chamber angles, without any underlying ocular or systemic conditions [14], [15]. In most cases, intraocular pressure (IOP) tends to rise above normal limits, reflecting a decrease in aqueous humor flow [16]. Although increased IOP is not the sole cause of optic nerve damage in POAG, it remains a major risk factor influencing disease progression. As research progresses, evidence increasingly suggests that oxidative and cytotoxic stress play an important role in the pathogenesis of glaucoma, where oxidatively altered proteins and their byproducts have the potential to be promising biomarkers for the detection and monitoring of this disease [17], [18].

Research by Mingxi Shao [12], emphasizes the role of bilirubin as a marker of oxidative and cytotoxic stress, which can affect both physiological and pathological processes in glaucoma. Shao's findings also indicate that total bilirubin (TBIL) levels have a specific association with primary open-angle glaucoma (POAG) based on gender, suggesting gender-based differences in susceptibility or disease progression related to oxidative stress.

This is in line with Dong et al. [13], report, which revealed that the relationship between TBIL and fundus arteriosclerosis differs between men and women. Specifically, high TBIL levels are significantly associated with fundus arteriosclerosis in men, while a similar correlation is not seen in women. Other studies also support these findings by showing that serum bilirubin concentrations are influenced by gender, with men tending to have higher average bilirubin levels than women.

In this study, all male subjects with POAG were in the advanced stage, so statistical analysis could not be performed due to data homogeneity. Among female subjects, 4 had moderate POAG and 15 had advanced POAG, with mean total bilirubin levels of 0.25 mg/dL (moderate) and 0.33 mg/dL (advanced), respectively. Independent T-test analysis showed no significant relationship between total bilirubin levels and POAG severity in women (p = 0.150). These findings are consistent with previous studies, such as Kim et al. [19], which showed different effects of bilirubin based on gender. Differences in results may also be influenced by sample size, measurement methods, or individual variability.

In addition, when compared to the control group, the average total bilirubin level in POAG subjects was 0.36 mg/dL, slightly lower than the control group, which had an average of 0.4 mg/dL. The Mann-Whitney test confirmed that this difference was not statistically significant (p=0.503).

Epidemiologically, POAG is estimated to occur in approximately 1 in 100 individuals over the age of 40, contributing to 12% of total blindness cases in countries such as the United Kingdom and the United States. The prevalence of this disease is known to increase with age. Deva's [20] study in Sanglah,

Denpasar, showed that the prevalence of POAG reached 44.6% in the 50-59 age group, while CIGTS data indicated that vision impairment due to POAG occurred about seven times more frequently in individuals over 60 years of age compared to those under 40 years of age. Consistent with these findings, this study shows that subjects over the age of 60 dominate both study groups, namely 76% in the POAG group and 80% in the control group, with no significant difference in age distribution (p=0.733).

Previous studies have reported differences in the prevalence of POAG based on gender, with some finding a higher prevalence in men [21], others finding a higher prevalence in women [22], while some studies found no significant difference [23].

In this study, female subjects dominated both groups, with 19 individuals (76%) in the POAG group and 15 individuals (60%) in the control group. Statistical analysis showed that the gender distribution did not differ significantly between the two groups (p=0.255), indicating that in this sample, gender was not a factor influencing the distribution of POAG.

Previous studies have shown that family history increases the risk of POAG [24], with genetic factors also contributing, as supported by genome-wide association studies [25]. In this study, of the 14 POAG subjects with a family history, the majority (12 individuals) were in the advanced stage with an average bilirubin level of 0.37 mg/dL, while 2 individuals were in the moderate stage with a level of 0.18 mg/dL. Independent T-test analysis showed no significant relationship between total bilirubin levels and POAG severity in this group (p=0.131). Similar results were found in subjects without a family history, where the average bilirubin level in the moderate stage was 0.32 mg/dL and in the advanced stage was 0.39 mg/dL, with the analysis results remaining insignificant (p=0.545).

Analysis based on age shows that in POAG subjects over 60 years of age, there were 3 people with moderate levels (average TBIL 0.23 mg/dL) and 16 people with advanced levels (average TBIL 0.37 mg/dL). with the Mann-Whitney test showing no significant relationship between total bilirubin levels and POAG severity (p=0.131). Meanwhile, in subjects aged 60 years and below, there were only 1 person with moderate severity and 5 people with advanced severity (TBIL 0.29 mg/dL vs 0.39 mg/dL), so statistical analysis could not be performed because the sample size in the moderate severity group was too small.

Overall, these findings indicate that total bilirubin levels are not significantly related to the severity of POAG, regardless of gender, family history, or age. These results confirm that although bilirubin is considered a potential biomarker of oxidative stress, in the population studied, TBIL may not be a sensitive indicator for assessing the severity of POAG. These findings also highlight the need for further research with larger sample sizes and consideration of more complex genetic and environmental factors.

4 **Conclusion**

The results of this study indicate that total serum bilirubin levels are not significantly associated with the severity of POAG, regardless of gender, age, or family history. These findings confirm that, although bilirubin plays a role as an endogenous antioxidant, its use as a clinical biomarker for assessing POAG progression or risk remains limited. Scientifically, this study adds evidence that a single antioxidant factor may not be sufficient to explain the complexity of POAG pathophysiology, and emphasizes the need for a multidimensional approach that considers the interaction between oxidative stress, genetic factors, and vascular mechanisms.

This study has several limitations, including a relatively small sample size and uneven distribution of subjects across some age, gender, and POAG severity categories, limiting statistical analysis to certain subgroups. Therefore, further research with larger samples, longitudinal designs, and additional oxidative biomarker measurements is needed to comprehensively evaluate the potential of bilirubin and other oxidative stress-related molecules as clinical indicators of POAG. These findings remain relevant as a basis for further research in developing early detection strategies and more personalized management for POAG patients.

Declaration of AI and AI assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT (OpenAI) to assist with language editing, grammar improvement, paraphrasing, and enhancing the clarity and readability of the manuscript. The AI tool was used solely to support the writing process and did not contribute to the study design, data collection, data analysis, interpretation of results, or the formulation of scientific conclusions. After using this tool, the authors carefully reviewed, revised, and verified all content and take full responsibility for the accuracy, integrity, and originality of the final manuscript.

Declaration of Competing Interest

The authors declare there is no competing interests.

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