

## Role of Serum Albumin-and Bilirubin Biomarkers in Glaucoma Patients

RUBIA AHMAD<sup>1</sup>, MARIA SARFARAZ<sup>2</sup>, MUHAMMAD FAISAL JAVAID<sup>3</sup>, ALIA SARFARAZ<sup>4</sup>, AHSAN FAROOQ<sup>5</sup>, MUHAMMAD NAZIR AWAN<sup>6</sup>

<sup>1</sup>Senior Registrar Radiology, Wah Medical College, Wah Cantt Rawalpindi

<sup>2</sup>Associate Professor Biochemistry, Rawal institute of Health Sciences Islamabad

<sup>3</sup>Assistant Professor Biochemistry Niazi Medical and Dental College Sargodha

<sup>4</sup>Assistant Professor Forensic Medicine, Islamabad Medical and Dental College Islamabad

<sup>5</sup>Associate Professor Radiology, Islamic International Medical College Railway Hospital, Rawalpindi

<sup>6</sup>Assistant Professor Anesthesia Islamic International Medical College Railway Hospital Rawalpindi

Corresponding author: Rubia Ahmad, Email: [subhan\\_89@hotmail.com](mailto:subhan_89@hotmail.com);

### ABSTRACT

**Objective:** To compared the levels of serum albumin (ALB) and bilirubin (BIL) in glaucoma patients to those of a control group of individuals who did not have the glaucoma.

**Methods:** A total of 50 glaucoma patients and 50 healthy controls were recruited from January-2022 to April-2022. Diagnosis of glaucoma was based on ophthalmology department. We recruited only those patients who were not having any kind of infection or were not taking antibiotics at the time of sample withdrawal. Serum ALB and BIL levels were measured in all patients.

**Results:** Mean age was 59.1±7.6 years in Glaucoma and 61.3±6.5 years in controls (p-value 0.12). There were 28 (56.0%) male patients in glaucoma and 27 (54%) male patients in controls (p-value 0.84). Mean serum albumin (ALB) levels were lower in glaucoma patients; 42.5±0.67 g/L versus 45.2±0.81 g/L in healthy controls (p-value <0.0001). Serum bilirubin levels were also lower in glaucoma patients; 15.1±1.80 mmol/L versus 18.3±1.95 mmol/L in healthy controls (p-value <0.0001).

**Conclusion:** Serum ALB and BIL are lower in glaucoma patients. However, ideal cut off values of these parameters are still to be decided to predict glaucoma.

**Keywords:** Albumin, Bilirubin, Glaucoma.

### INTRODUCTION

Glaucoma is a neurodegenerative disorder with complicated aetiology that primarily damage the optic nerve (ON) and retinal ganglion cells (RGC), resulting in permanent vision loss.<sup>1</sup> Despite the fact that increasing intraocular pressure (IOP) is the most important worry, the exact process of glaucoma damage is uncertain. Oxidative stress, vascular factors, and neuroinflammation are other responsible factors.<sup>2,3</sup> Oxidative stress has been established as a critical etiologic component in the development of glaucoma. There has been a reported imbalance between antioxidant defence activities and prooxidative state.<sup>4</sup>

The current lack of success in the early diagnosis and prevention of glaucoma has encouraged medical professionals to explore for innovative treatments by making use of sophisticated biotechnological processes. Animal models are being used in an increasing number of research projects on glaucoma. These studies provide valuable insight into the biochemical pathways and potential targets that are affected by this disease, but they are not always appropriate for use in noninvasive patient examinations.<sup>5</sup>

In clinical biochemical testing, sometimes known as regular hospital examinations in clinical practise, serum albumin (also abbreviated as ALB) and bilirubin (also abbreviated as BIL) are traditionally considered to be two indications of hepatobiliary function. It is important to note that both of them have been shown to possess potent antioxidant activities and have the potential to act as serum indicators in conditions linked with oxidative stress and inflammation.<sup>6</sup> In the presence of oxidative stress, ALB undergoes a transformation that leaves it in an oxidised state. This oxidised status is undetectable by conventional laboratory procedures, which results in patients having lower serum levels of ALB. On the other hand, high amounts of BIL have been proven to produce neurotoxicity as well as oxidative stress; yet, studies conducted in the field of molecular-biology have demonstrated that bilirubin itself is an effective antioxidant. In neurodegenerative disorders, it has been reported that serum ALB and BIL levels are significantly decreased.<sup>7,8</sup> However, the exact nature of the relationship between glaucoma and serum ALB, or BIL is still mainly a mystery.

In this study, we compared the levels of serum ALB and BIL in glaucoma patients to those of a control group of individuals who did not have the eye disease.

### MATERIAL AND METHODS

The study was conducted in Biochemistry department, Wah Medical College, Wah Cantt Rawalpindi. We enrolled 50 glaucoma patients who had been referred to the biochemistry laboratory for a full blood profile investigation. Prior to participation in the experiment, all participants were thoroughly informed of the risks and benefits and submitted an informed consent form. Ophthalmologists diagnosed glaucoma based on ocular examinations, glaucomatous clinical symptoms, age and family history. Patients having secondary glaucoma, other eye disorders, no hepatobiliary disease, or hematopoietic system disease or no coagulation abnormalities, having systemic diseases, or any other neurodegenerative disorders (such as Parkinson's) are excluded.

Healthy controls were consenting adults with no history of eye disease or glaucoma who took part in routine health screenings during the course of the trial.

When patients were being evaluated for their diagnosis, blood cell counts and serum biochemical testing were performed. CPT tubes that were anticoagulated with EDTA were used to collect blood from the peripheral veins. In order to obtain blood samples, serum separator tubes were utilised. At the time of the blood collection, the patients were not suffering from any acute infections nor were they taking any medications that had the potential to influence either the blood cell components or the serum biochemistry profiles. The serum ALB and BIL levels were measured using an Architect C 16000 (Abbott) instrument at the biochemistry department of the hospital.

Prism 8.4 was used to conduct the statistical analysis. An independent sample t-test was used to examine the statistical differences between the groups.

### RESULTS

Mean age was 59.1±7.6 years in Glaucoma and 61.3±6.5 years in controls (p-value 0.12). There were 28 (56.0%) male patients in glaucoma and 27 (54%) male patients in controls (p-value 0.84) [Table 1].

Mean serum albumin (ALB) levels were lower in glaucoma patients; 42.5±0.67 g/L versus 45.2±0.81 g/L in healthy controls (p-value <0.0001). Serum bilirubin levels were also lower in glaucoma patients; 15.1±1.80 mmol/L versus 18.3±1.95 mmol/L in healthy controls (p-value <0.0001) [Table 2].

Table 1: Baseline Variables.

	Glaucoma patients (N=50)	Healthy Controls (N=50)	P-value
Age	59.1±7.6	61.3±6.5	0.12
Sex			
Female	28 (56%)	27 (54%)	0.84
Male	22 (44%)	23 (46%)	

Table 2: Comparison of ALB and BIL Levels.

	Glaucoma patients (N=50)	Healthy Controls (N=50)	P-value
ALB (g/L)	42.5±0.67	45.2±0.81	<0.0001
BIL (mmol/L)	15.1±1.80	18.3±1.95	<0.0001

## DISCUSSION

The relevance of oxidative and antioxidative state in the aetiology of glaucoma has been demonstrated both in patients and in animal models. The pathological situation known as oxidative stress takes place when the antioxidant capacity of the body is surpassed by an excessive amount of reactive oxygen species (ROS). This can result in the death of cells as well as a buildup of apoptotic residues, the production of autoantibodies, and the activation of the autoimmune cascade response. The amino acid bundle (ALB) test evaluates not only the capacity of the liver to produce protein but also its nutritional state. Because of the persistent presence of oxidative stress in plasma, ALB plays an essential role as an antioxidant in this fluid. Because it acts as a "tramp steamer" in the circulation, ALB has the potential to absorb a broad variety of substances, some of which are significant ROS-generators when they react with oxygen.<sup>9</sup> These compounds include transition metals (such as iron or copper) and polyunsaturated fatty acids. The ability of this compound to form bonds with ligands is responsible for a diverse array of antioxidant effects. In spite of the fact that the large amount of ALB in the serum and its rapid clearance from the circulation have been regarded as "biologically insignificant," evidence suggests that ALB's antioxidant capacity may be impaired in many pathological conditions, including diabetes and chronic kidney disease.<sup>10</sup> This is the case despite the fact that these two factors have been regarded as "biologically insignificant." Oxidized ALB, along with Alzheimer's disease and Parkinson's disease, has been connected to an elevated risk of oxidative stress in recent years. There have been studies conducted on the redox status of ALB in glaucoma patients. It has been suggested that the redox condition of vitreous ALB can serve as a biomarker for determining the redox status of the vitreous in POAG patients.<sup>11,12</sup> Ischemia-modified albumin levels are much higher in people who have PACG, which suggests that oxidative stress may play a role as a biomarker.<sup>13</sup> According to these findings, patients who suffer from glaucoma have lower serum ALB concentrations than healthy controls do.

In spite of the fact that BIL is traditionally thought of as a byproduct of heme degradation and that it is cytotoxic at high concentrations, recent evidence shows that this iron porphyrin metabolite has a number of biological properties, including antioxidative, anti-inflammatory, and neuroprotective properties at physiological concentrations.<sup>14</sup> This is the case even though BIL is considered to be a byproduct of heme degradation and that it is cytotoxic at high concentrations. The ability of BIL to scavenge reactive oxygen species (ROS) and decrease NADPH oxidase activity may be the basis for its antioxidant effects. According to studies conducted in clinical settings, serum BIL levels are also related with cardiovascular disorders.<sup>15,16</sup> Few studies have focused on the role that BIL plays in the pathogenesis of glaucoma, despite the rising evidence that oxidative stress and vascular factors play a significant role in the development of glaucoma. According to the findings of our study, glaucoma patients had lower levels of both TBIL and IBIL than healthy controls did.

Recent study suggests that both retinal local neuroinflammation and systemic inflammation may play a role in

the aetiology of glaucoma.<sup>17</sup> As a result of this, measures of systemic inflammation have been proposed for use as indicators in the diagnosis of sickness as well as the prediction of clinical outcomes. Peripheral venous blood can be utilised for screening and diagnosis in high-risk patients because it is inexpensive, simple, widely available, and suited for cellular and molecular investigation in tiny volumes. These characteristics make it an ideal specimen for these types of examinations (even a few millilitres). It is simple to examine blood markers while searching for indications of systemic inflammation. Some of these blood markers include white blood cells (WBC), neutrophils, and the ratio of neutrophils to lymphocytes (NLR). Biomarkers such as these have proven to be quite useful in the early identification and individual screening of glaucoma. In those who have PACG, the number of white blood cells (WBC), neutrophils, neutrophil-like cells (NLR), and platelet-like cells (LMR) all rise significantly, while the number of lymphocytes, platelets, and LMR all fall. It has been demonstrated that NLR is capable of providing an accurate representation of the severity of the disease.<sup>18</sup> In recent years, blood cell and serum biochemical indices such as NAR have emerged as valuable indicators of systemic inflammation. These indices have also been employed in the treatment of inflammatory and vascular illnesses as well as cancer. An increase in the amount of NAR in a patient's blood has been linked to an increase in the patient's likelihood of passing away from cardiogenic shock (CS). The diagnostic sensitivity of NAR is significantly higher than the individual levels of neutrophils in the blood or albumin in the serum.<sup>19</sup> According to the findings of an experiment carried out by Varim and his colleagues, patients who had an infection with COVID-19 and NAR had a worse prognosis and a higher fatality rate. NAR has been associated to the delayed start of cerebral ischemia that might occur following an aneurysmal subarachnoid haemorrhage.<sup>20</sup>

## CONCLUSION

Serum ALB and BIL are lower in glaucoma patients. However, ideal cut off values of these parameters are still to be decided to predict glaucoma.

## REFERENCES

1. Tham YC, Li X, Wong TY, Quigley HA, Aung T, Cheng CY. Global prevalence of glaucoma and projections of glaucoma burden through 2040: a systematic review and meta-analysis. *Ophthalmology*. 2014;121(11):2081-90.
2. Williams PA, Marsh-Armstrong N, Howell GR; Lasker/IRRF Initiative on Astrocytes and Glaucomatous Neurodegeneration Participants. Neuroinflammation in glaucoma: A new opportunity. *Exp Eye Res*. 2017;157:20-27.
3. Tezel G, Yang X, Luo C, Kain AD, Powell DW, Kuehn MH, et al. Oxidative stress and the regulation of complement activation in human glaucoma. *Invest Ophthalmol Vis Sci*. 2010;51(10):5071-82.
4. Kimura A, Namekata K, Guo X, Noro T, Harada C, Harada T. Targeting Oxidative Stress for Treatment of Glaucoma and Optic Neuritis. *Oxid Med Cell Longev*. 2017;2017:2817252.
5. Ban N, Siegfried CJ, Apte RS. Monitoring Neurodegeneration in Glaucoma: Therapeutic Implications. *Trends Mol Med*. 2018;24(1):7-17.
6. Das S, Maras JS, Hussain MS, Sharma S, David P, Sukriti S, et al. Hyperoxidized albumin modulates neutrophils to induce oxidative stress and inflammation in severe alcoholic hepatitis. *Hepatology*. 2017 Feb;65(2):631-646.
7. Kim JW, Byun MS, Lee JH, Yi D, Jeon SY, Sohn BK, et al; KBASE Research Group. Serum albumin and beta-amyloid deposition in the human brain. *Neurology*. 2020;95(7):e815-e826.
8. Li J, Zhao L, Wang Z, Zhao X, Wu J. Association of Serum Indirect Bilirubin Concentrations with Motor Subtypes of Parkinson's Disease. *Neurodegener Dis*. 2019;19(3-4):155-162.
9. Roche M, Rondeau P, Singh NR, Tarnus E, Bourdon E. The antioxidant properties of serum albumin. *FEBS Lett*. 2008;582(13):1783-7.
10. Michelis R, Sela S, Zeitun T, Geron R, Kristal B. Unexpected Normal Colloid Osmotic Pressure in Clinical States with Low Serum Albumin. *PLoS One*. 2016;11(7):e0159839.

11. Schwab C, Paar M, Fengler VH, Lindner E, Haas A, Ivastinovic D, et al. Vitreous albumin redox state in open-angle glaucoma patients and controls: a pilot study. *Int Ophthalmol*. 2020;40(4):999-1006.
12. Karakurt Y, Mertoglu C, Gok G, Ucak T, Tasli N, Icel E, et al. Thiol-Disulfide Homeostasis and Serum Ischemia Modified Albumin Levels in Patients with Primary Open-Angle Glaucoma. *Curr Eye Res*. 2019;44(8):896-900.
13. Chang D, Sha Q, Zhang X, Liu P, Rong S, Han T, et al. The evaluation of the oxidative stress parameters in patients with primary angle-closure glaucoma. *PLoS One*. 2011;6(11):e27218.
14. Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN. Bilirubin is an antioxidant of possible physiological importance. *Science*. 1987;235(4792):1043-6.
15. Schwertner HA, Jackson WG, Tolan G. Association of low serum concentration of bilirubin with increased risk of coronary artery disease. *Clin Chem*. 1994;40(1):18-23.
16. Perlstein TS, Pande RL, Beckman JA, Creager MA. Serum total bilirubin level and prevalent lower-extremity peripheral arterial disease: National Health and Nutrition Examination Survey (NHANES) 1999 to 2004. *Arterioscler Thromb Vasc Biol*. 2008;28(1):166-72.
17. Li S, Qiu Y, Yu J, Shao M, Li Y, Cao W, et al. Association of systemic inflammation indices with visual field loss progression in patients with primary angle-closure glaucoma: potential biomarkers for 3P medical approaches. *EPMA J*. 2021;12(4):659-675.
18. Tang B, Li S, Han J, Cao W, Sun X. Associations between Blood Cell Profiles and Primary Open-Angle Glaucoma: A Retrospective Case-Control Study. *Ophthalmic Res*. 2020;63(4):413-22.
19. Peng Y, Xue Y, Wang J, Xiang H, Ji K, Wang J, et al. Association between neutrophil-to-albumin ratio and mortality in patients with cardiogenic shock: a retrospective cohort study. *BMJ Open*. 2020;10(10):e039860.
20. Zhang X, Liu Y, Zhang S, Wang C, Zou C, Li A. Neutrophil-to-Albumin Ratio as a Biomarker of Delayed Cerebral Ischemia After Aneurysmal Subarachnoid Hemorrhage. *World Neurosurg*. 2021;147:e453-e458.