**Original Research Article**

**EVALUATION OF ANTIOXIDANTS, AND HAEMATOLOGICAL INDICES IN GLAUCOMA PATIENTS: A CASE STUDY OF BAYELSA STATE, NIGERIA**

**ABSTRACT**

**Introduction**: Glaucoma is a neurodegenerative eye condition and is characterized by raised intraocular pressure. When left untreated, patients may gradually experience visual field loss, and even lose their sight completely.

**Aim**: This study aimed to evaluate plasma enzymatic and nonenzymatic antioxidants levels and haematological parameters in glaucoma patients in Bayelsa State, Nigeria.

**Methods**: A cross-sectional study design was used in this investigation to examine the relationship between variables, and a total of 80 subjects comprising; 50 glaucoma patients and 30 apparently healthy control subjects selected for the study. Following standard operating protocols, five milliliters of venous blood were aseptically drawn from each patient via the cubital fossa region and dispensed in ethylene diamine tetra-acetic acid and plain sample containers. The blood sample was used for determination of antioxidants: uric acid, albumin, bilirubin, Superoxide dismutase, catalase, and malondialdehyde using spectrophotometric techniques, and haematological parameters: packed cell volume, haemoglobin, total white blood cell count, neutrophil, lymphocytes, eosinophils, basophils, monocytes, and platelet count were measured using the Sysmex automated haematological analyzer. Statistical analysis was done using Special Package for Social Sciences (SPSS) version 23.0 and p<0.05 was considered statistically significant.

**Results**: The serum concentration of non-enzymatic antioxidants (uric acid, albumin, total bilirubin and conjugated bilirubin) levels in the glaucoma patients were significantly (p<0.05) lower than the non-glaucomatous subjects. Serum superoxide dismutase, catalase and malondialdehyde levels showed no significant difference when compared with the control subjects. Packed cell volume, haemoglobin, total white blood cell count, and platelets showed no significant (p>0.05) difference in the glaucoma patients compared to the control subjects. However, a statistically significant decrease in eosinophil count (p<0.05) was observed in glaucoma patients compared to control subjects which indicated a significant association between glaucoma and eosinopenia.

**Conclusion**: This finding suggests that the reduced uric acid, albumin, total bilirubin, conjugated bilirubin and eosinophil levels may be a potential risk for glaucoma.

**Keywords:** Antioxidants, Glaucoma, Haematological Indices, Malondialdehyde

**INTRODUCTION**

Glaucoma is a disorder that results in progressive neuropathy in the visual field and is characterized by structural alterations to the optic disk or optic nerve head (Srivastav *et al*., 2024). If left unmanaged, it can result to an irreversible or complete vision loss (Tham et al., 2014). A report in 2020 indicate that glaucoma affected about 80 million individuals, and it is projected to increase to 111.8 million by 2040, affecting a larger population in Asian and African regions (Tham et al., 2014). Glaucoma is one of the topmost causes of blindness globally, and it is the second most common cause of blindness in Nigeria, with an incidence of 19% (Akinlabi et al., 2009).

Glaucoma is classified into two primary types: open-angle glaucoma and closed-angle glaucoma. These types are subdivided into primary open angle glaucoma, primary angle closure glaucoma and secondary glaucoma (He et al., 2006). Primary open-angle glaucoma is the most widespread type of glaucoma, which is characterized by a gradual obstruction of drainage channels that raises intraocular pressure and gradually damages the optic nerve (Douglass et al., 2023). Primary angle-closure glaucoma occurs when the iris bends forward, creating direct contact with the trabecular meshwork, which blocks the outflow of aqueous humor from the eye. The secondary glaucoma arises as a result of another illness like eye injuries, pigmentary dispersion syndrome, uveitis, or drugs like corticosteroids and cycloplegics that raises intraocular pressure, and damages optic nerve which impair vision (Sena et al., 2017)

The development of glaucoma is influenced by multiple factors and the exact pathways and mechanisms are not yet fully understood. Nonetheless, it has been demonstrated that several contributing risk factors, including vascular alterations, age, race, raised glutamate levels, corneal thickness, elevated intraocular pressure, and genetic variables, play a major role to the development of glaucoma ((Akinlabi et al., 2009; Zhang et al., 2011). Microvascular injury can result from hyper-viscosity brought on by alterations of blood cells and components of plasma. Impaired nitric oxide metabolism, endothelial dysfunction, neuroinflammation and vasospasm have been implicated as possible mechanisms of glaucoma (Astafurov et al., 2014).

Primary haematological disorders are usually uncommon, while haematological manifestations due to other diseases like diabetes, hypertension, renal disorders, cancers among others occur regularly. Haemorrhagic glaucoma is a medical complication of vascular disorder that is highly associated with glaucoma development and progression (Lee et al., 2021). Haematological indices are measurable components of blood like red blood cells, white blood cells and platelets, which originate from the haemopoeitic stem cell (Azuonwu et al., 2017). The evaluation of haematological indices plays a crucial role in healthcare, as some serve as essential indicators for assessing anaemia, leukaemia, haemophilia and immune function, guiding treatment decisions, and monitoring disease progression and therapeutic outcomes (Amilo et al., 2024). To ensure accurate diagnosis and effective patient management, these indices are routinely analyzed (Amilo et al., 2024). Over the past decade, the understanding of glaucoma's causes has shifted from a solely pressure-based theory to an integrated mechanical and vascular perspective (Delaney et al., 2006). Alterations in blood cell properties and plasma components leading to hyper-viscosity may contribute to microvascular damage (Mannini et al., 2007). Additionally, factors such as reduced nitric oxide availability, endothelial dysfunction, and vasospasms are increasingly associated with glaucoma (Delaney et al., 2006).

Oxidative stress is thought to be a major etiological component that becomes increasingly important in the development of glaucoma (Goyal et al., 2013). According to report by Hsueh et al., (2022) and Kimura et al. (2017), an imbalance between oxidative stress and antioxidant defense activity contributes to the development of glaucoma and other ocular diseases. Sacca et al., (2020) reported that oxidative stress may have an impact on the retinal ganglion cells and other retinal cells in the posterior part of the eye. It may also cause degeneration of the human trabecular meshwork in the anterior part of the eye, which raises intraocular pressure and sets off the glaucoma pathogenetic cascade (Zhao et al., 2017).

Antioxidants (enzymatic and non-enzymatic), function as defense mechanisms for ocular tissues against oxidative stress (Hsueh et al., 2022). They may help protect against glaucoma through various mechanisms, including lowering intraocular pressure, supporting vascular health, and preventing the loss of retinal ganglion cells (Jabbehdari et al., 2021). Key enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase, while non-enzymatic antioxidants such as uric acid, albumin, vitamin C, and vitamin E play a crucial role as major ocular antioxidants (Wojcik et al., 2013). According to Das et al. (2017), bilirubin and albumin have antioxidant properties and may be used biomarkers for disorders associated with oxidative stress. An imbalance between oxidative stress and the body's antioxidant defenses plays a role in the development of various eye diseases. As a result, antioxidants may serve as biomarkers for prognosis and as potential therapeutic targets for managing conditions linked to oxidative stress (Umapathy et al., 2013). Abu-Amero and colleagues, (2013) and Abu-Amero and colleagues, (2014) have shown a decrease in total antioxidant status (TAS) among glaucoma patients.

It has been reported that antioxidants like albumin, bilirubin, and uric acid have demonstrated a significant reduction in neurodegenerative diseases, but the nexus between serum uric acid, or albumin or bilirubin and glaucoma is unclear (Qin et al., 2015). Several authors have evaluated the role of antioxidants in glaucoma taking into consideration a strong link between oxidative stress and the development of glaucoma. However, the results obtained by different authors have shown contradicting reports. Also, there are only few studies carried out on the assessment of haematological indices in glaucoma with contradicting results. This study aimed to evaluate plasma enzymatic and nonenzymatic antioxidants levels and haematological parameters in glaucoma patients.

**2.0 MATERIALS AND METHODS**

### 

### **2.1 Study Design and Subjects**

The study utilized a cross-sectional study design, which was carried out between July, 2022 and May, 2023. The study comprises fifty (50) male and female subjects diagnosed with glaucoma, who visited the Ophthalmology Department, Niger Delta University Teaching Hospital (NDUTH) Okolobiri, Bayelsa State, between July, 2022 and May, 2023. Inclusion criteria of this study include; subjects diagnosed with any type of glaucoma, glaucoma patients without a known metabolic disorder, male and female subjects who consented to the study and are within the age of 18 – 65 years. Exclusion criteria include; glaucoma patients with a known chronic metabolic, haematological and immunological disorder like diabetes, hypertension, coagulopathy, anemia, liver and renal diseases were excluded. Chronic smokers and alcoholics, and subjects who do not consent to the study were also excluded. The study also included a control group of individuals who were apparently healthy. The control subjects selected for this study had no history of ocular diseases and underwent the same examinations as the patients. They were also not on any medication and were non-smokers. Prior to the study, ethical approval was obtained from the Research and Ethical Committee of Niger Delta University Teaching Hospital, Okolobiri, Yenagoa, Bayelsa State. Additionally, informed consent was obtained from all participants before their enrollment.

**2.2 Collection of Blood Samples**

Venous blood samples (5ml) were collected from all subjects via the cubital fossa region following standard operation procedures. The blood was drawn into plain sample containers and ethylene diamine tetra-acetic acid (EDTA) container. The blood samples in the plain containers were allowed to stand to clot properly for 1 hour at room temperature. The clot was retracted and the blood was centrifuged at 5000 rpm for 15 minutes. The supernatant (serum) obtained was aspirated into a separate labeled plain sample container. The samples were assayed within 1 hour for the biochemical parameters; Uric acid, albumin, bilirubin, Superoxide dismutase, Catalase (CAT), and Lipid peroxidation product malondialdehyde using spectrophotometric techniques. The blood samples in the EDTA container were analyzed within 1 hours for haematological indices using automated haematology analyzer (Mindray Auto Haematology Analyzer, HM-500X, 2016, Germany)

**2.3 Determination of Haematological Indices**

Hematological indices were analyzed using an automated hematology analyzer (Mindray Auto Hematology Analyzer, HM-500X, 2016, Germany).

Principle: The Beckman Coulter method, which is used for particle sizing and counting, relies on detecting changes in electrical resistance caused by non-conductive particles suspended in an electrolyte solution. As a suspension of blood cells passes through a narrow orifice alongside an electric current, each cell creates an impedance change proportional to its size. The system then counts individual cells and generates a size distribution profile. To enhance accuracy, the number of cells counted per sample is approximately 100 times greater than a conventional microscopic count, reducing statistical error by nearly tenfold.

Procedure: The hematology analyzer was powered on, and the sample ID was entered and verified on the touchscreen display. The blood sample was gently mixed by inverting the tube at least three times. The sample tube was then positioned for aspiration by the analyzer’s probe. Once aspirated, the machine processed the sample, displayed the results on the screen, and printed them out for further analysis [10].

**2.4 Determination of Enzymatic and Non-enzymatic Antioxidants**

Serum superoxide dismutase (SOD) levels were assessed by the direct colorimetric method using spectrophotometer as described by Okutu and Onitsha, (Okutu et al., 2022). Principle: This method is based on the generation of superoxide radicals from xanthine and xanthine oxidase, which interact with 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (MilliporeSigma (Sigma-Aldrich Merck): Offers INT with a purity of 95%.), resulting in the formation of a red formazan dye.

Serum catalase (CAT) activity was determined following the direct colorimetric method outlined by Okutu and Onitsha (2022). Principle: This technique relies on the reduction of dichromate to chromic acetate in the presence of hydrogen peroxide (H₂O₂) when heated. The chromic acetate produced is then measured spectrophotometrically at 570 nm.

Serum malondialdehyde (MDA) concentration was estimated using a spectrophotometric method as described by Onitsha and Okutu (2021). Principle**:** MDA forms a conjugate with thiobarbituric acid (TBA), which is separated and measured. Trichloroacetic acid (TCA) (Cayman Chemical's TBARS Assay Kit) is used to precipitate serum proteins, which are then removed by centrifugation. The MDA-TBA complex, which appears pink, is measured at 534 nm.

Total bilirubin (TB) and conjugated bilirubin (CB) were using the Jendrassik and Groff method (Jendrassik and Groff, 1938). Principle**:** Sulfanilic acid is converted into a highly reactive diazonium salt (Sigma-Aldrich (Merck Millipore); sulfanilic acid diazonium salt and 4-nitrobenzenediazonium tetrafluoroborate) through its reaction with nitrous acid, which is generated from sodium nitrite and hydrochloric acid. Conjugated bilirubin (water-soluble bilirubin diglucuronide) reacts with diazotized sulfanilic acid to produce red azobilirubin. Upon adding alkaline tartrate reagent, the red azobilirubin is transformed into a blue azobilirubin with higher absorbance.

Uric acid levels were determined using the Uricase-PAP enzymatic method (Gochman & Schmitz , 1971). Principle: The enzyme uricase converts uric acid into allantoin and hydrogen peroxide. The hydrogen peroxide then reacts with a phenolic compound and 4-aminophenazone in the presence of peroxidase, forming a red quinoneimine dye complex. The intensity of this complex is directly proportional to the uric acid concentration in the sample.

Serum albumin levels were measured using the bromocresol green (BCG) method (Sigma-Aldrich's BCG Albumin Assay Kit (MAK124), as described by Laitinen and Kolthoff (1939). Principle**:** Bromocresol green, a yellow pH indicator at 3.5–4.2, binds specifically to albumin, forming a blue-green complex. The absorbance of this complex is measured at 632 nm, with the intensity of the color being directly proportional to the albumin concentration in the sample.

**2.5 Statistical Analysis**

The collected data were analyzed using the Statistical Package for Social Sciences (SPSS) version 23. Results were presented in tables for clarity. A Student's t-test was conducted to compare the mean values between the test and control groups. Additionally, descriptive statistics such as frequency, mean, standard deviation, and standard error were calculated, along with an unpaired t-test for each parameter. Statistical significance was set at P < 0.05.

**3.0 RESULTS**

Table 1 shows that the serum concentration of uric acid, total bilirubin, conjugated bilirubin, and albumin were significantly (p<0.05) lower in the glaucoma patients than the control subjects. The serum activities of catalase and superoxide dismutase in the glaucoma patients were slightly lower than the control group, but was not significant (p>0.05). The mean value of serum malondialdehyde (MDA) was slightly elevated in glaucoma patients than the control subjects, but was not significant (p>0.05).

Table 2 shows that the average age of glaucoma patients was significantly higher (P < 0.05) than that of the control group. The mean values of packed cell volume (PCV), haemoglobin (Hb), total white blood cell (WBC) count, lymphocytes, neutrophils, monocytes, and platelets counts showed no significant difference (p>0.05) between the glaucoma patients and non-glaucoma subjects (control). However, the mean value of eosinophil count was significantly lower (P < 0.05) in glaucoma patients than the control groups.

Table 3 indicates that serum concentration of uric acid (U/A), total bilirubin (TB), and conjugated bilirubin (CB) levels were significantly higher (P < 0.05) in male glaucoma patients compared to female patients. However, albumin (ALB), superoxide dismutase (SOD), and malondialdehyde (MDA) showed no significant difference (P > 0.05) between male and female patients.

**Table 1: Comparison of Mean Values of Serum Antioxidants and Malondialdehyde Levels in Glaucoma Patients and Non-glaucomatous Subjects Under Study**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameter | Non-Glaucoma n=30 (X±SD) | Glaucoma n=50  (X±SD) | P-Value | Remark |
| U/A (mmol/L | 0.32 ± 0.12 | 0.17 ± 0.06 | 0.001\* | S |
| TB (µmol/L | 8.77 ± 2.31 | 7.25 ± 2.11 | 0.027\* | S |
| CB (µmol/L | 3.53 ± 1.02 | 2.38 ± 1.31 | 0.010\* | S |
| ALB (g/L) | 54.2 ± 4.33 | 40.3 ± 3.03 | 0.001\* | S |
| CAT (µl/L) | 56.1 ± 3.74 | 52.5 ± 3.21 | 0.171 | NS |
| SOD (µl/L) | 59.3 ± 4.31 | 52.9 ± 4.01 | 0.135 | NS |
| MDA (nmol/ml) | 3.85 ± 1.21 | 4.01 ± 1.24 | 0.352 | NS |

Results were represented in mean and standard deviation. Student T-test was used for comparing the groups. Keys: S=Significant; NS=Non-significant; MDA=Malonaldehyde; TB=Total bilirubin; CB=Conjugated bilirubin; CAT=Catalase; SOD=Superoxide Dismutase; ALB= Albumin; U/A=uric acid.

**Table 2: Comparison of the Mean Values of Haematological Indices of Glaucoma Patients and Non-glaucomatous Subjects Under Study**

Haematological Glaucoma Patients Non- Glaucoma Patients P- Remark (n=50) (n=30) value

Age (years) 38.33 ± 6.34 33.53 ± 5.32 0.003\* S

PCV (%) 40.5 ± 1.07 41.5 ± 2.23 0.762 NS

Hb (g/dl) 12.78 ± 1.07 12.99 ± 2.23 0.642 NS

TWBC (x 109/L) 7.21 ± 1.07 6.65 ± 1.23 0.182 NS

NEUT (%) 47.35 ± 7.13 44.02 ± 6.04 0.856 NS

LYMP (%) 49.02 ± 5.13 49.05 ± 5.21 0.256 NS

MONO (%) 1.72 ± 0.12 1.99 ± 0.14 0.504 NS

EOSIN (%) 1.02 ± 1.08 4.45 ± 1.21 0.002\* S

PLT(x109/l) 203.45 ± 20.22 231.48 ± 21.43 0.378 NS

Key: Values marked with an asterisk (\*) indicate statistical significance at P < 0.05. Results are presented as Mean ± Standard Deviation (SEM). A Student’s t-test was used for analysis, with P < 0.05 considered statistically significant. 'NS' denotes non-significant results, while 'S' indicates statistical significance. Abbreviations: TWBC: Total White Blood Cell count, NEUT: Neutrophil, LYMP: Lymphocyte, MONO: Monocyte, EOSIN – Eosinophil, and PLT – Platelet.

**Table** **3: Comparison of Serum Antioxidants and Malondialdehyde levels in Glaucoma patients by Gender.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameter | Male (n=21)  (X±SD) | Female (n=29)  (X±SD) | P-Value | Remark |
| U/A (mmol/L | 0.37 ± 0.11 | 0.24 ± 0.02 | 0.001 | S |
| TB (µmol/L | 8.81 ± 2.23 | 8.07 ± 2.01 | 0.022 | S |
| CB (µmol/L | 3.68 ± 1.42 | 3.17 ± 1.04 | 0.045 | S |
| ALB (g/L) | 40.9 ± 4.00 | 40.7 ± 4.13 | 0.720 | NS |
| CAT (µl/L) | 45.1 ± 3.11 | 45.3 ± 3.21 | 0.241 | NS |
| SOD (µl/L) | 54.1 ± 4.01 | 53.9 ± 4.33 | 0.132 | NS |
| MDA(nmol/ml) | 4.06 ± 1.20 | 4.02 ± 1.10 | 0.751 | NS |

Results were represented in mean and standard deviation. Student T-test was used for comparing the groups. Keys: S=Significant, NS=Non-significant, MDA=Malonaldehyde, TB=Total bilirubin, CB= Conjugated bilirubin, CAT=Catalase, SOD=Superoxide Dismutase, ALB=Albumin, U/A=Uric acid.

**4.0 DISCUSSION**

Glaucoma is a heterogenous and multifactorial neurodegenerative disease and is characterized by raised intraocular pressure. When left untreated, patients may gradually experience visual field loss, and even lose their sight completely (Steinmetz et al., 2022). A systematic review by Tham et al., (2014) reports that the prevalence of glaucoma is rising and varies globally, and it is projected that the number of people with glaucoma worldwide may increase to 111.8 million in 2040, disproportionately affecting people residing in Asia and Africa (Tham et al., 2014). In Nigeria, glaucoma is reported as the second leading cause of blindness with incidence rate of 19%. Unlike cataract (23%), which is the leading cause of blindness it has no known cure (Akinlabi and Iyawe, 2007).

Antioxidants function as defense mechanisms for ocular tissues against oxidative stress (Hsueh et a., 2022). The non-enzymatic antioxidants such as uric acid, albumin, bilirubin, vitamin C, and vitamin E are considered as the main ocular antioxidant’s molecules (Wojcik et al., 2013). According to Das and colleagues, (2017), bilirubin and albumin have antioxidant properties and may be utilized as blood biomarkers for disorders linked to oxidative stress. Previous research has highlighted a decline in serum bilirubin and albumin levels in individuals suffering from neurodegenerative and neuroinflammatory conditions, including multiple sclerosis and Parkinson’s disease (Liu et al., 2008). Erdurmus et al. (2019) also reported a reduction in total antioxidant capacity in patients with primary open-angle glaucoma and pseudoexfoliation glaucoma. Similarly, our study observed a significant decrease in serum albumin, total bilirubin, and conjugated bilirubin levels among glaucoma patients compared to the control group. These findings align with studies by Chong et al. (2022) and Rubia et al. (2022). The results suggest that elevated bilirubin and albumin levels could be linked to intraocular pressure (IOP) regulation and bilirubin-related neurological dysfunction.

Uric acid (UA) plays a vital role as an antioxidant, possessing metal-chelating properties and the ability to neutralize nitrogen radicals and superoxide, thereby preventing the formation of potent oxidants like peroxynitrite (Li et al., 2016). It has been proposed that UA may provide neuroprotection against oxidative stress-related damage (Bowman et al., 2010; Irizarry et al., 2009). In this study, the serum concentration of uric acid in the glaucoma patients were significantly (P<0.05) lower than the control groups. According to Tanito et al. (2012), a decrease in serum UA may contribute to oxidative stress insufficiency, which could be a factor in glaucoma progression. However, this observation contrasts with findings from Yuki et al. (2020) and Li et al. (2019), who reported elevated UA levels in glaucoma patients compared to healthy individuals. Li et al. (2016) found that glaucoma patients had significantly lower UA levels, while Al-Khateeb et al. (2015) documented those individuals with lower baseline UA levels had a higher likelihood of glaucoma progression over time. These results indicate that reduced serum UA levels may serve as a risk factor for glaucoma. Conversely, Yang et al. (2022) found that higher UA levels were associated with reduced retinal capillary plexus vessel density, suggesting a potential detrimental effect on the retinal microvasculature and underscoring the need to regulate UA levels to prevent vascular changes.

Antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GST), play a crucial role in mitigating oxidative stress by eliminating harmful by-products and protecting cells from free radical-induced damage (Wojcik et al., 2013). Previous studies have demonstrated a reduction in total antioxidant capacity in glaucoma patients, however, this study revealed no significant difference (p>0.05) in serum SOD and CAT activities between the glaucoma patients and control groups, though a slight reduction in SOD and CAT was observed in the glaucoma patients. Malondialdehyde (MDA), an oxidative stress biomarker formed from the peroxidation of polyunsaturated fatty acids such as arachidonic acid, is typically measured as Thiobarbituric Acid Reactive Substances (TBARS). In this study, the MDA levels showed no significant difference (p>0.05) between the glaucoma patients and the control group, but was slightly higher in the glaucoma patients than the control subjects. These results are in agreement with Hasan and colleagues, (2017), who indicated that no significant variation in serum SOD, CAT and MDA levels. However, it contradicts the findings of Ferreira et al., (2004), which indicated a significant increase in SOD and glutathione peroxidase activity in glaucoma patients, and no significant changes in CAT activities.

The present study also observed that the serum concentration of uric acid, total bilirubin, and conjugated bilirubin in the male glaucoma patients were significantly higher than the female patients (P<0.05). This result contradicts the previous study of Octavia *et al.,* (2012), who reported that women have stronger antioxidant potential than men, and this could be due to the protective effects of estrogen, which may enhance resistance to oxidative stress.

Previous studies have shown contradicting reports on haematological indices in glaucoma patients. Gwyn et al., (1933) reported that inflammatory cell counts change little with advancing age in primary glaucoma patient. Akinlabi and Iyawe, (2007) reported reduction of eosinophil (eosinopenia) in glaucoma patients, and no significant difference observed in Packed Cell Volume, total and differential white blood cell count. In the present study, there was no significant difference observed in Packed Cell Volume, heamoglobin, total White blood cell, differential white blood cell count and platelet count between glaucoma patients and control. However, a significant (p<0.05) reduction in eosinophil (eosinopenia) observed in the glaucoma patients in comparison with the control subjects. The reduction in the eosinophil count could be attributed to the use of certain medications such as steroids (dexamethasone) which is known to exert a direct survival-inhibitory effect on eosinophil, while it induces elevated intraocular pressure (IOP) (Bartlett *et al*., 1993). It has been reported that both eosinophil count and IOP respond to diurnal variation. Eosinophil count is highest at (mid) night during sleep, lowest in the morning and rises by mid-afternoon. In reverse order IOP has been indicated to be lowest at about midnight, highest in the morning and decreases by mid-afternoon. Adrenocorticosteroids hormones (ACTH) affects both eosinophil count and IOP but in opposite direction (Akinlabi and Iyawe, 2007).

Another possible mechanism for the reduction in eosinophil (eosinopenia) could be due to stress which is known to cause reduction in eosinophil count, while elevation of intraocular pressure (IOP) was associated with anxiety, anger and depression and a lower IOP occurs when the patient was relatively happy and relaxed Akinlabi and Iyawe, (2007). The eosinopenia observed could also be attributed to prostaglandin which enhances activation of eosinophil, and prostaglandin analog latanoprost is the current drug of use in lowering intraocular pressure (IOP) (Mannini *et al.,* 2007).

**5.0 Conclusion**

This study has demonstrated a significant decline in serum albumin, total bilirubin, conjugated bilirubin, and uric acid levels in glaucoma patients compared to healthy individuals. These findings indicate that oxidative stress exposure alters antioxidant levels, potentially contributing to increased intraocular pressure and optic nerve damage, which could ultimately result in vision loss. Additionally, the observed relationship between glaucoma and eosinophil levels indicate that eosinopenia could be a potential risk factor for the disease. Further large-scale investigations are recommended to establish a more definitive link between antioxidants and eosinophil levels and glaucoma.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

We hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

**CONSENT**

A written informed consent was obtained from all participants before their enrollment into the study

**ETHICAL APPROVAL**

Prior to the study, ethical approval was obtained from the Research and Ethical Committee of Niger Delta University Teaching Hospital, Okolobiri, Yenagoa, Bayelsa State.

**COMPETING INTERESTS DISCLAIMER:**

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

**REFERENCES**

Abu-Amero, K. K., Azad, T. A., Mousa, A., Osman, E. A., Sultan, T., et al. (2014). Total antioxidant level is correlated with intraocular pressure in patients with primary angle closure glaucoma. *BMC Research Notes, 7*, 163.

Abu-Amero, K. K., Kondkar, A. A., Mousa, A., Osman, E. A., & Al-Obeidan, S. A. (2013). Decreased total antioxidants in patients with primary open-angle glaucoma. *Current Eye Research, 38*, 959–964.

Ahmad, R., Sarfaraz, M., Javaid, M. F., Sarfaraz, A., Farooq, A., & Awan, M. N. (2022). Role of serum albumin- and bilirubin biomarkers in glaucoma patients. *Pakistan Journal of Medical & Health Sciences, 16*(5), 501–509.

Akinlabi, G. A., & Iyawe, V. I. (2007). Haematological Parameters in Open Angle Glaucoma Patients. *Journal of Medicine and Biomedical Research*, **6**(1-2), 35-40.

Al-Khateeb, E., Althaher, A., Al-Khateeb, M., Al-Musawi, H., Azzouqah, O., Al-Shweiki, S., & Shafagoj, Y. (2015). Relation between uric acid and Alzheimer’s disease in elderly Jordanians. *Journal of Alzheimer’s Disease, 44*, 859–865.

Amilo, D., Izuchukwu, C., Sadri, K., Yao, H. R., Hincal, E., et al. (2024). A fractional- order model for optimizing combination therapy in heterogeneous lung cancer: Integrating immunotherapy and targeted therapy to minimize side effects. *Scientific Reports, 14*(1), 18484.

Astafurov, K., Elhawy, E., Ren, L., Dong, C. Q., Igboin, C., Hyman, L., et al. (2014). Oral microbiome link to neurodegeneration in glaucoma. *PloS one*, *9*(9), e104416

Azuonwu, O., Nnenna, I., & Uwuma, O. E. (2017). Evaluation of haematological profile of geriatric subjects in Port Harcourt metropolis of Niger Delta of Nigeria. *Journal of Clinical Laboratory Medicine, 2*(1), 23-28.

Bartlett, J. D., Horwitz, B., Laibovitz, R., & Howes, J. F. (1993). Intraocular pressure response to loteprednol etabonate in known steroid responders. *Journal of Ocular Pharmacology and Therapeutics, 9*(2), 157–165

Bowman, G. L., Shannon, J., Frei, B., Kaye, J. A., & Quinn, J. F. (2010). Uric acid as a CNS antioxidant. *Journal of Alzheimer’s Disease, 19*, 1331–1336.

Das, S., Maras, J. S., Hussain, M. S., Sharma, S., David, P., Sukriti, S., Shasthry, S. M., Maiwall, R., Trehanpati, N., Singh, T. P., & Sarin, S. K. (2017). Hyperoxidized albumin modulates neutrophils to induce oxidative stress and inflammation in severe alcoholic hepatitis. *Hepatology, 65*(2), 631–646.

Delaney, Y., Walshe, T. E., & O’Brien, C. O. (2006). Vasospasm in glaucoma: Clinical and laboratory aspects. *Optometry and Vision Science, 83*(7), 406–414.

Douglass, A., Dattilo, M., & Feola, A. J. (2023). Evidence for menopause as a sex-specific risk factor for glaucoma. *Cellular and molecular neurobiology*, *43*(1), 79-97.

Ferreira, S. M., Lerner, S. F., Brunzini, R., Evelson, P. A., & Llesuy, S. F. (2004). Oxidative stress markers in aqueous humor of glaucoma patients. *American Journal of Ophthalmology, 137*(1), 62–69.

Gochman, N., & Schmitz, J. M. (1971). Automated determination of uric acid, with use of a uricase-peroxidase system. *Clinical Chemistry, 17*(12), 1154–1159.

Goyal, A., Srivastava, A., Sihota, R., & Kaur, J. (2014). Evaluation of oxidative stress markers in aqueous humor of primary open-angle glaucoma and primary angle closure glaucoma patients. *Current Eye Research, 39*(8), 823–829.

Gwyn, D. R., Stewart, W., Hennis, H. L., McMillan, T. A., & Pitts, R. A. (1993). The influence of age upon inflammatory cell count and structure in chronic open-angle glaucoma. *Acta Ophthalmologica (Copenhagen), 71*(5), 691–695.

Hasan, A., Garg, P., Chandra, A., & Gupta, M. (2017). Relationship between oxidative stress and primary open-angle glaucoma. *Journal of Evidence-Based Medicine and Healthcare, 4*(82), 4830–4834.

He, C., Zhang, G., Fu, J., Zhang, R., Li, A., Liu, D., Li, B., Chen, Y., Deng, B., Chen, Y., & Shuai, P. (2022). Clinical significance of albumin- and bilirubin-based biomarkers in glaucoma: A retrospective case-control study. *Oxidative Medicine and Cellular Longevity, 2022*, 8063651.

He, M., Foster, P. J., Ge, J., Huang, W., Zheng, Y., Friedman, D. S., et al. (2006). Prevalence and clinical characteristics of glaucoma in adult Chinese: a population-based study in Liwan District, Guangzhou. *Investigative ophthalmology & visual science*, *47*(7), 2782- 2788.

Hsueh, Y. J., Chen, Y. N., Tsao, Y. T., Cheng, C. M., Wu, W. C., & Chen, H. C. (2022). The patho-mechanism, antioxidant biomarkers, and treatment of oxidative stress-related eye diseases. *International Journal of Molecular Sciences, 23*(3), 1255.

Irizarry, M. C., Raman, R., Schwarzschild, M. A., Becerra, L. M., Thomas, R. G., Peterson, R. C., Ascherio, A., & Aisen, P. S. (2009). Plasma urate and progression of mild cognitive impairment. *Neurodegenerative Diseases, 6*, 23–28.

Jabbehdari, S., Chen, J. L., & Vajaranant, T. S. (2021). Effect of dietary modification and antioxidant supplementation on intraocular pressure and open-angle glaucoma. *European Journal of Ophthalmology, 31*, 1588–1605.

Jendrassik, L., & Groff, P. (1938). Colorimetric method for measurement of bilirubin. *Biochemical Journal, 297*(81), 160–166.

Kimura, A., Namekata, K., Guo, X., Noro, T., Harada, C., & Harada, T. (2017). Targeting oxidative stress for treatment of glaucoma and optic neuritis. *Oxidative Medicine and Cellular Longevity, 2017*, 2817252.

Laitinen, H. A., & Kolthoff, I. M. (1939). A study of diffusion processes by electrolysis with microelectrodes. *Journal of the American Chemical Society, 61*(12), 3344–3349.

Lee, E. J., Jung, H. J., Jong, C. H., & Changwon, K. (2021). Evidence-based understanding of disc hemorrhage in glaucoma. *Survey of Ophthalmology, 66*(3), 412–422

Li, S., Shao, M., Cao, W., & Sun, X. (2019). Association between pretreatment serum uric acid levels and progression of newly diagnosed primary angle‐closure glaucoma: A prospective cohort study. *Oxidative Medicine and Cellular Longevity, 2019*(1), 7919836.

Li, S., Shao, M., Tang, B., Zhang, A., Cao, W., & Sun, X. (2016). The association between serum uric acid and glaucoma severity in primary angle closure glaucoma: A retrospective case- control study. *Oncotarget, 8*(2), 2816.

Liu, Y., Li, P., Lu, J., Xiong, W., Oger, J., et al. (2008). Bilirubin possesses powerful immunomodulatory activity and suppresses experimental autoimmune encephalomyelitis. *The Journal of Immunology, 181*(3), 1887–1897.

Mannini, L., Cecchi, E., Fatini, C., Marcucci, R., Alessandriello, A., et al. (2007). Clinical hemorheology and microcirculation. *Annali dell'Istituto Superiore di Sanità, 43*(2), 144–150.

Octavia, Y., Tocchetti, C. G., Gabrielson, K. L., Janssens, S., Crijns, H. J., et al. (2012). Doxorubicin-induced cardiomyopathy: From molecular mechanisms to therapeutic strategies. *Journal of Molecular and Cellular Cardiology, 52*(6), 1213–1225.

Okutu, J. B., & Enebrayi, O. (2022). Ameliorative effect of *Allium sativum* and *Justicia carnea* extracts co-administration on acute cadmium chloride-induced changes on liver function parameters of albino rats. *World Journal of Pharmacy and Life Sciences, 4*, 11–24.

Onitsha, E. N., & Okutu, J. B. (2021). Influence of vitamin E and selenium on reproductive hormones and lipid peroxidation levels in lead-induced toxicity in female Wistar rats. *IOSR Journal of Environmental Science, Toxicology, and Food Technology, 15*(2), 1–9.

Qin, X. L., Zhang, Q., Sun, H. M. W., & Hu, Z. T. (2015). Lower serum bilirubin and uric acid concentrations in patients with Parkinson's disease in China. *Cell Biochemistry and Biophysics, 72*(1), 49–56.

Saccà, S. C., Vernazza, S., Iorio, E. L., Tirendi, S., Bassi, A. M., et al. (2020). Molecular changes in glaucomatous trabecular meshwork: Correlations with retinal ganglion cell death and novel strategies for neuroprotection. *Progress in Brain Research, 256(1):*151-188.

Sena, D. F., & Lindsley, K. (2017). Neuroprotection for treatment of glaucoma in adults. *Cochrane Database of Systematic Reviews*, (1).

Steinmetz, J. D., Bourne, R. R., Briant, P. S., Flaxman, S. R., Taylor, H. R., et al. (2021). Causes of blindness and vision impairment in 2020 and trends over 30 years, and prevalence of avoidable blindness in relation to VISION 2020: The Right to Sight: An analysis for the Global Burden of Disease Study. *The Lancet Global Health, 9*(2), e144–e160.

Srivastav, Y., Taj, B., Singh, J., Yadav, S., and Ahmad, M.I. (2024). Glaucoma (Eye Disease) and its Associated Diagnosis and Treatment Process: A Schematic Concise Review. *Ophthalmology Research: An International Journal,* 19(3):42-50.

Tanito, M., Kaidzu, S., Takai, Y., & Ohira, A. (2012). Status of systemic oxidative stresses in patients with primary open-angle glaucoma and pseudoexfoliation syndrome. *PLoS One, 7*(11), e49680.

Tham, Y. C., Li, X., Wong, T. Y., Quigley, H. A., Aung, T., & Cheng, C. Y. (2014). Global prevalence of glaucoma and projections of glaucoma burden through 2040: a systematic review and meta-analysis. *Ophthalmology*, *121*(11), 2081-2090.

Tosun, M., Yağcı, R., & Erdurmuş, M. (2019). Glaucoma and antioxidant status. In *Handbook of Nutrition, Diet, and the Eye* (pp. 203–219). Academic Press.

Umapathy, A., Donaldson, P., & Lim, J. (2013). Antioxidant delivery pathways in the anterior eye. *Biomedical Research International, 2013*, 207250.

Wojcik, K. A., Kaminska, A., Blasiak, J., Szaflik, J., & Szaflik, J. P. (2013). Oxidative stress in the pathogenesis of keratoconus and Fuchs endothelial corneal dystrophy. *International Journal of Molecular Sciences, 14*, 19294–19308.

Yang, K., Li, C., Shi, K., Zhu, X., Xiao, Y., Su, B., et al. (2022). Association of serum uric acid with retinal capillary plexus. *Frontiers in Endocrinology, 13*, 855430.

Yuki, K., Asaoka, R., Ono, T., Awano-Tanabe, S., Murata, H., & Tsubota, K. (2020). Evaluation of fear of falling in patients with primary open-angle glaucoma and the importance of inferior visual field damage. *Investigative Ophthalmology & Visual Science, 61*(3), 52.

Zhang, S. H., Dong, F. T., Mao, J., & Bian, A. L. (2011). Factors related to prognosis of refractory glaucoma with diode laser transscleral cyclophotocoagulation treatment. *Chinese Medical Sciences Journal*, *26*(3), 137-140.

Zhao, J., Wang, S., Zhong, W., Yang, B., Sun, L., & Zheng, Y. (2016). Oxidative stress in the trabecular meshwork (Review). *International Journal of Molecular Medicine, 38*, 995– 1002.