ORIGINAL ARTICLE

Comparison of Reactive Oxygen Species in the Blood with Oxidant Antioxidant Imbalance in the Aqueous Humor of Type 2 Diabetic Cataract Patients: A Cross-Sectional Study in Rawalpindi

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ABSTRACT

Objective: To compare the total antioxidant capacity, total oxidant status, and oxidative stress index in the aqueous humor of type 2 diabetic and non-diabetic cataract patients and determine their correlation with the glycemic control and serum Malondialdehyde levels.

Study Design: Cross-sectional analytical study.

Place and Duration of Study: This study was conducted at the Department of Physiology, Pak Emirates Military Hospital (PEMH), Rawalpindi, Pakistan, from 1st June 2022 to 31st January 2023.

Methods: This cross-sectional analytical study recruited 120 subjects, who were categorized into two equal groups. Aqueous humor was collected before cataract surgery to measure the total oxidant status and the total antioxidant capacity by spectrophotometry. Fasting glucose, glycosylated hemoglobin and malondialdehyde levels were measured in the blood. The Statistical Package for the Social Sciences (SPSS) version 24 was used to analyze the results. A *P* value ≤ 0.05 was considered significant.

Results: The fasting blood glucose, glycosylated hemoglobin, and malondialdehyde levels were significantly raised in the diabetic group compared to the non-diabetic group. Similarly, total oxidant status and oxidative stress index were significantly increased in the aqueous humor of diabetic patients, while the total antioxidant capacity was reduced compared to non-diabetics. Fasting blood glucose and malondialdehyde levels were positively correlated with total oxidant status and oxidative stress index, but negatively correlated with the total antioxidant capacity in aqueous humor.

Conclusion: Increased oxidative stress in the blood of type 2 diabetic patients leads to an increase in the total oxidant status and a decrease in the total antioxidant capacity in the AH. Moreover, poor glycemic control also adversely contributes to this imbalance, which is significantly more compared to the otherwise healthy individuals having cataract. Therefore, an imbalance of the total oxidant status and the total antioxidant capacity in the aqueous humor is suggested to play a significant role in the development of cataract in type 2 diabetes mellitus patients.

Keywords: *Aqueous Humor, Cataract, Oxidative Stress, Type 2 Diabetes Mellitus.*

How to cite this: *Farooq A, Aleem S. Comparison of Reactive Oxygen Species in the Blood with Oxidant Antioxidant Imbalance in the Aqueous Humor of Type 2 Diabetic Cataract Patients: A Cross-Sectional Study in Rawalpindi. Life and Science. 2026; 7(2): 225-230. doi: http://doi.org/10.37185/LnS.1.1.814*

Introduction

Cataract refers to the diminished transparency of the crystalline lens due to opacity, standing out as one of the primary causes of early blindness globally.[1] The onset of cataracts in individuals with type 2 diabetes mellitus (T2DM) is believed to stem from heightened inflammation in the anterior eye chamber and blood vessels throughout the body. Identifying these risk factors becomes crucial for the prevention or early initiation of treatment to mitigate vision loss caused by cataracts.[2] A survey conducted in three districts of

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Received: Sep 18, 2024; 1st Revision Received: May 29, 2025
2nd Revision Received: Dec 13, 2025; Accepted: Jan 11, 2026

the Punjab province in Pakistan revealed an overall occurrence rate of 15.3% for senile cataracts in individuals aged 30 and above, while it was 4.3% across all age groups in a sample of 1,269 individuals.[3] Notably, the incidence of age-related cataracts in the Pakistani population exhibited a gender difference, with a higher prevalence in men (48%) compared to women (39%) among those aged 70 and above.[4]

For individuals with T2DM under the age of 65, the prevalence of cataracts is three to four times higher. Persistent hyperglycemia and insufficient metabolic control are recognized as primary risk factors for cataract development.[5] The Beaver Dam Eye Study determined the association between poor glycemic control and higher incidence, along with a faster progression of cataracts in T2DM patients.[6] Several oxidative stress markers, like lipid peroxidation products and oxidized glutathione increase in the blood and aqueous humor of T2DM patients, along with decrease in antioxidants like ascorbate, taurine, superoxide dismutase, catalase, and glutathione peroxidase.[7,8] This causes osmotic and oxidative stress in the lens caused by the intracellular accumulation of sorbitol, the depletion of glutathione due to NADPH degradation, and excessive fructose metabolites production by the polyol pathway due to increased flux of glucose.[6]

Developing countries endure substantial health damage and subsequent economic setbacks due to T2DM and cataracts. In these regions, T2DM treatment is frequently insufficient, and cataract surgery remains often financially unattainable.[9] Hence, exploring the connection between these two diseases becomes imperative to minimize their adverse impacts on the Pakistani population. Measuring the total oxidant status (TOS) and total antioxidant capacity (TAC) of the aqueous humor is a practical and appropriate method to assess the magnitude of stress on the lens compared to the study of individual antioxidant or oxidant molecules as seen in various studies.[10]

The aim of our study was to compare the TOS, TAC, and OSI in the aqueous humor of cataract patients with and without T2DM, and their correlation with blood glucose, HbA1c, and MDA levels.

Methods

This study was conducted at the Department of Physiology, Pak Emirates Military Hospital (PEMH), Rawalpindi, Pakistan, from 1st June 2022 to 31st January 2023 after taking approval from the Institutional Ethical Review Committee of the institute, vide letter no: ERC/ID/206, dated 30th May 2022. A total of 120 cataract patients, including both genders, aged 55-65 years, were enrolled and divided into two equal groups. Group I comprised of non-diabetic while Group II included T2DM patients. The sample size was determined using an online sample size calculator with a confidence interval of 95%. Patients with ophthalmic conditions other than cataract (such as glaucoma, uveitis, prior ocular surgery, etc.), systemic illness, those taking vitamin A, C, E supplements, non-steroidal anti-inflammatory drugs, smokers, and alcoholics/narcotics were excluded.[5]

Upon obtaining informed consent, all subjects undertook a thorough ophthalmological examination, including slit lamp biomicroscopy and stereoscopic ocular fundus evaluation to rule out other ocular diseases. A detailed medical history and general physical examination were conducted. Cataract was evaluated based on its hardness as per the Lens Opacities Classification System III.[11] Data, including age, gender, duration of T2DM, fasting blood glucose (FBG), eye disorders, and medications, were recorded on a separate proforma.

After an overnight fast, 5ml of blood was drawn from the antecubital vein to determine FBG using the hexokinase method on the ADVIA 1800 clinical chemistry system (Randox glucose hexokinase, Randox Laboratories, UK). HbA1c levels in blood samples were measured using ADVIA 1800, which detects turbidity produced by anti-HbA1c antibodies. Serum MDA levels were determined using the MDA kit (catalog no. AP8428, Bio Assay technology), employing a sandwich enzyme-linked immunosorbent assay (ELISA) principle with antibodies and Streptavidin-Horseradish Peroxidase Enzyme (HRP), and measuring absorbance at 450 nm.[12]

Before any conjunctival or intraocular intervention during cataract surgery, 0.1–0.2 ml of aqueous humor was extracted using a 26-gauge insulin syringe. The aqueous humor samples were stored at

-80°C till biochemical analysis was conducted at the Center of Research, Education, and Applied Medicine Laboratory of the Army Medical College, Rawalpindi.

TAC was measured by using the Glory science kit (Cat AP001, Lot 1511470231) by ELISA. The assay relies on the oxidation of the ferrous ion-o-dianisidine complex by oxidants in the sample to form ferric ion, leading to color changes. Spectrophotometry measures color intensity, and the results are expressed as millimolar Trolox equivalents per liter (mmol Eqv./L).[13]

TOS was measured in the aqueous humor using the Elabscience kit (Cat E-BC-K802-M, Lot 1010876302) by ELISA. Oxidants in the sample oxidize ferrous to ferric ions in an acidic environment. The ferric ion binds highly with xylenol, producing a blue-purple complex. The depth of the color, proportionate to the amount of oxidants, is measured at a wavelength near 590 nm, permitting the indirect calculation of the total oxidation state of the solution.[13]

Data analysis was employed using IBM SPSS (Statistical Package for the Social Sciences) version 25. Quantitative variables, including TAC, TOS, OSI ratio, FBG, HbA1c, and MDA, were measured. TAC and TOS were expressed as mmol H2O2 Eqv./L, FBG as mg/dl, HBA1c as mmol/mol, and MDA as mmol/L (OSI, being a ratio, has no specific units). The independent sample student t-test compared groups, and the Pearson correlation coefficient assessed relationships between numerical variables. A *P*-value ≤0.05 was considered significant.

Results

An independent-samples t-test was conducted to compare the mean values between the two cohorts. Analysis revealed that the diabetic group exhibited statistically significant elevations in plasma concentrations of FBG, HbA1c, and MDA compared to the control group ($P < 0.001$) (Table 1). Within the aqueous humor, the diabetic group demonstrated a significantly higher Total Oxidant Status (TOS) and a significantly lower Total Antioxidant Capacity (TAC), culminating in a statistically significant increase in the Oxidative Stress Index (OSI) (Table 2).

Pearson's product-moment correlation was employed to assess the relationship between systemic and ocular biomarkers. Results indicated that plasma FBG, HbA1c, and MDA levels were

Table 1: Comparison of FBG, HbA1c and serum MDA levels between group 1 and group 2

Blood parameters	Group 1 (non-diabetic) Mean ± SD	Group 2 (diabetic) Mean ± SD	*t* - value	*P*- value
FBG (mg/dl)	91.61 ± 5.71	109.183 ± 12.32	10.016	0.001
HbA1c (mmol/mol)	5.43 ± 0.57	6.46 ± 0.85	7.716	0.001
Serum MDA Levels (mmol/litre)	332.39 ± 124.25	673.00 ± 115.35	15.562	0.001

Table 2: Comparison of TAC, TOS, and OSI in the aqueous humor of cataract patients in group 1 and group 2

Aqueous humor parameters	Group 1 Mean ± SD	Group 2 Mean ± SD	*t* value	*P*- value
TAC (mmol H_2O_2 Eqv./L)	1.01 ± 0.21	0.367 ± 0.19	-17.102	0.001
TOS (mmol H_2O_2 Eqv./L)	6.83 ± 1.74	9.67 ± 2.56	7.082	0.001
OSI = TOS/TAC*	7.05 ± 2.39	32.93 ± 17.07	11.627	0.001

Oxidative Stress Index (OSI), Total Oxidant Status (TOS), Total Antioxidant Capacity (TAC)

Table 3: Correlation of TAC, TOS, and OSI with duration of T2DM, fasting blood glucose, HbA1c, and serum MDA levels

	Total Antioxidant Capacity (mmol H_2O_2 EqV./L)	Total Oxidative Stress (mmol H_2O_2 EqV./L)	Oxidative stress Index OSI (arbitrary units)
Fasting glucose (mg/dl)	-6.87	0.427	0.726
HbA1c (mmol/ml)	-4.74	0.320	0.531
Serum MDA levels (mmol/L)	-7.18	0.364	0.560

Oxidative Stress Index (OSI), Total Oxidant Status (TOS), Total Antioxidant Capacity (TAC)

positively correlated with aqueous TOS and OSI, while showing a significant inverse correlation with aqueous TAC. (Table 3). Post hoc sensitivity analysis confirmed that the study was adequately powered, with statistical power exceeding 0.80, thereby minimizing the risk of a Type II error.

Discussion

Prolonged exposure to hyperglycemia and the disruption of oxidant-antioxidant balance contribute significantly to oxidative stress in T2DM.[14] Our work is consistent with studies by Hou Y et al. and Chitra PS et al., who also observed the rise in plasma MDA levels due to hyperglycemia, reflecting oxidative damage to body lipids driven by increased non-enzymatic and auto-oxidative glycosylation in T2DM patients.[15,16] Increased plasma glucose and HbA1c were positively correlated with MDA levels, signifying elevated oxidative stress in T2DM patients. Reactive oxygen species primarily target lipids, leading to lipid peroxidation of cellular structures.[12] A positive correlation of MDA with HbA1c observed in our study is also consistent with previous studies.[15,16]

The glucose concentration in healthy aqueous humor is around 3.2 mm and it is derived from plasma. The lens uptakes this glucose for metabolic purposes through specific glucose transporters present in its membrane.[6] Hyperglycemia and increased oxidative stress observed in the plasma of diabetic patients also impact their aqueous humor, as indicated by positive correlations between glycemic control markers and oxidative stress indicators in aqueous humor, along with negative correlations with antioxidants.[17] Various pathological processes give rise to different oxidative stress markers in the diabetic eye, and the relevance of oxidative stress in aqueous humor to cataract development surpasses that of plasma.[18] While studies on aqueous humor oxidative stress have often focused on individual markers, this study suggests that assessing total oxidative stress provides a more comprehensive indicator of patient damage.

In measuring TAC, TOS, and OSI in aqueous humor, this study found that T2DM elevates TOS and reduces TAC levels compared to non-diabetic cataract patients. This suggests that, in addition to the osmotic stress, T2DM strongly affects TOS and TAC levels of aqueous humor, which in turn impact the lens.[10] Similar results were observed by Atalay E et al. and Yilmaz M et al., indicating high TOS and OSI along with low TAC in aqueous humor of diabetics.[19,20] This significant rise in TOS and OSI in the aqueous humor of the diabetic group indicates an intensified oxidative stress on the lens in diabetics. The higher oxidative stress in diabetic cataract patients may be attributed to increased glucose concentration in the aqueous humor, leading to glycation of lens proteins and subsequent production of sorbitol. Sorbitol accumulation induces osmotic stress, generating free oxygen radicals, promoting oxidation and disulfide bond formation in lens proteins, and increasing the risk of cataract development.[6]

Reduced antioxidant capacity in the aqueous humor of diabetics, as shown in similar studies, implies a role in cataract pathophysiology.[5,15,19,20] Elevated plasma glucose and HbA1c levels were found to reduce the antioxidant levels in aqueous humor. TAC levels, positively correlated with uric acid, albumin, glutathione, and other antioxidants, decreased in the diabetic group due to increased antioxidant consumption counteracting rising oxidant levels.[18,19]

The study revealed significant negative correlations between plasma glucose, HbA1c, and serum MDA levels and TAC levels in the aqueous humor, suggesting that poor glycemic control may increase ocular oxidative stress, potentially contributing to earlier cataract onset.[8] Similar results were obtained by Hou Y et al. Atalay E et al. and Yilamz M et al.[15,19,20] The findings support the idea that diabetic patients experience increased oxidant levels and diminished antioxidant capacity in aqueous humor, possibly contributing to cataract progression.[21] The observed correlation between oxidative stress in plasma and aqueous humor underscores their interconnectedness, as aqueous humor is derived from blood plasma.

However, it's important to note that the impact of disease distribution, compliance, and treatment mode on study results remains unknown due to varied patient profiles. While optimal blood glucose regulation is suggested to prevent or delay cataract development by maintaining low oxidative stress in the aqueous humor and comparable antioxidant levels, future studies are needed to establish the intricate relationship among glycemic control, oxidative stress, and cataract development.[21,22]

Conclusion

The high oxidative stress in the blood of type 2 diabetic patients with cataract correlates to an elevated oxidative stress and a reduced total antioxidant capacity in the aqueous humor. This oxidant and antioxidant imbalance significantly contributes to cataract development. Moreover, poor glycemic control exacerbates this imbalance, which further contributes to the adverse conditions conducive to cataract formation.

Acknowledgment: The authors are grateful for the guidance and support of the administration, ophthalmologists, and staff at AFIO in collecting samples and gathering relevant information from the patients included in this study. We are especially thankful to Dr. Teyyeb Azeem Janjua for his pivotal role in helping the authors with this research.

Conflict of Interest: The authors declare no conflict of interest

Grant Support and Financial Disclosure: None

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Author Contributions

AF: Conception, design of the work, validation of data, interpretation, write-up of results, revising, editing, supervising for intellectual content, and approval for final submission

SA: Manuscript writing for methodology design, investigation, data acquisition, curation, statistical analysis, writing the original draft, proofreading, and approval for final submission

AF is the nominated guarantor and takes full responsibility for the overall content and integrity of the work