Clinical Ophthalmology

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ORIGINAL RESEARCH

Downregulation of TRPV4 and LRRC8A in Crystalline Lenses of Diabetic Patients May Contribute to Cataractogenesis

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Background: Type 2 diabetes mellitus (T2DM) is associated with increased cataract risk, potentially due to osmotic imbalance from intracellular sorbitol accumulation affecting lens cell volume regulation.

Objective: To evaluate the expression of Kir4.1, TRPV4, and LRRC8A channels—key regulators of cell volume—in the anterior lens capsule of patients with and without T2DM.

Methods: A cross-sectional observational study was conducted using anterior lens capsules from 50 patients undergoing cataract surgery (24 with T2DM, and 26 non-diabetic). Cataract severity was graded using the Lens Opacities Classification System III, and immunofluorescence was used to quantify protein expression.

Results: T2DM patients exhibited greater severity in cortical and nuclear cataracts, particularly among women. Diabetic samples showed increased Kir4.1 and decreased TRPV4 and LRRC8A expression.

Conclusion: The study identifies associations between T2DM and altered expression of osmoregulatory proteins in the lens capsule, which may relate to the formation and severity of cataracts. These exploratory findings warrant further investigation to clarify underlying mechanisms.

Plain Language Summary: Diabetes mellitus is associated with a higher risk of developing cataracts, although the underlying mechanisms are not fully understood. This study examined lens tissue from patients with and without type 2 diabetes who underwent cataract surgery. We focused on three proteins—Kir4.1, TRPV4, and LRRC8A—that help regulate cell volume and osmotic balance in the lens.

Using fluorescent labeling, we compared the expression of these proteins between the two groups. All three were present in diabetic and non-diabetic samples, but TRPV4 and LRRC8A showed lower expression levels in patients with diabetes. These differences may reflect changes in the lens environment associated with diabetes and cataract severity.

These findings provide preliminary insights into how osmoregulatory proteins might be involved in lens changes observed in diabetes. Further research is needed to clarify their role and potential relevance for future therapeutic strategies.

Keywords: cataract, diabetes mellitus type 2, Kir4.1, TRPV4, LRRC8A

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by high blood glucose levels, known as hyperglycemia.[1] Around 589 million adults aged 20 to 79 worldwide live with diabetes, representing nearly 11% of this population.[1] DM is primarily classified into two main types: type 1 diabetes mellitus (T1DM), caused by an autoimmune process that destroys pancreatic β-cells, leading to complete insulin deficiency,[2] and type 2 diabetes mellitus (T2DM), which involves insulin resistance and/or a relative deficiency in insulin secretion.[3] Although its exact cause is

Received: 1 August 2025
Accepted: 11 November 2025
Published: 17 December 2025

not fully understood, it is strongly associated with being overweight. T2DM is the most common form, making up over 90% of all diabetes cases worldwide.[1] The eye is one of the organs most commonly affected by DM, with ocular complications such as cataract formation,[4] diabetic retinopathy,[5] and glaucoma,[6] impairing vision and remaining the leading causes of blindness.[7]

The lens is a transparent structure composed of highly organized fiber cells enveloped by a membranous envelope known as the lens capsule.[8] Different pathological insults can cause the denaturation and clumping of crystallin proteins within these fibers, leading to the loss of lens transparency.[8,9] Cataract, characterized by opacification of the lens or its capsule, blocks light transmission to the retina, significantly affecting visual quality.[10] This condition leads to reduced visual acuity, glare, halos around lights, and photophobia, and it is one of the leading causes of blindness worldwide.[10,11] The main risk factors for developing cataracts include advanced age, prolonged exposure to ultraviolet light, smoking, and systemic conditions such as diabetes. Additional contributing factors are chronic corticosteroid use, family history, ocular trauma, and previous ocular surgeries.[12]

In DM, elevated glucose levels are observed in the aqueous humor of the anterior chamber of the eye, which promotes the passive diffusion of glucose into the lens. Once inside the lens, glucose is converted to sorbitol via the aldose reductase pathway.[13,14] The intracellular accumulation of sorbitol creates a hyperosmotic state, leading to increased water influx into the lens fibers, resulting in significant swelling of the cortical fibers and the formation of lens opacities.[15]

Osmotic cell volume regulation is regulated by two mechanisms that balance changes in cell volume.[16] Regulatory Volume Decrease (RVD) occurs when cell volume increases in a hypotonic environment, leading ions and osmolytes to exit the cell, followed by water loss to restore the cell's original size. Conversely, Regulatory Volume Increase (RVI) happens when cells shrink in a hypertonic environment, prompting the uptake of solutes and water to regain normal cell volume.[17–19] Changes in ion channels involved in RVD and RVI during DM may directly contribute to cellular osmotic stress, thereby worsening the effects of intracellular sorbitol buildup.

In this study, we analyzed the alterations of the RVD mechanism in the lens capsules of patients with DM by examining the expression of the ion channels: TRPV4 (Transient Receptor Potential Cation Channel Subfamily V Member 4), Kir4.1 (Inwardly rectifying potassium channel 4.1), and LRRC8A (Leucine-rich repeat-containing protein 8A), which are directly involved in osmotic control.

Purpose

To evaluate the expression levels and distribution patterns of the osmotic-regulating proteins Kir4.1, TRPV4, and LRRC8 in the anterior lens capsules of cataract patients, comparing individuals with and without T2DM.

Patients and Methods

Patients and Study Design

This prospective, cross-sectional, observational, and descriptive study was conducted at the Unidad de Investigación APEC-UNAM, Hospital "Dr. Luis Sánchez Bulnes" of the Asociación Para Evitar la Ceguera en México, I.A.P. (APEC). The study period spanned from August 2022 to May 2024. The research complied with the ethical principles outlined in the Declaration of Helsinki and was approved by the Institutional Review Board (IRB) and the Ethics and Research Committee of the Association to Prevent Blindness in Mexico under the number INV-22-03. All participants were fully informed about the study's objectives and procedures and provided written informed consent before enrollment. The confidentiality and anonymity of patient data were strictly maintained throughout the study.

The target population comprised Mexican patients aged ≥18 years with cataracts, with T2DM (study group) or without diabetes (control group), who were candidates for phacoemulsification cataract surgery at the APEC. Patients with a history of ocular inflammation, trauma, or intraocular surgery, as well as those who did not receive treatment at the study hospital, were excluded. A total of 50 anterior capsule samples were collected: 24 from T2DM patients and 26 from non-diabetic patients (control group). Demographic information, including age and sex, was obtained from all participants. For patients with T2DM, fasting capillary glucose levels and glycated hemoglobin (HbA1c) values were recorded.

Preoperative cataract diagnosis and classification were performed using the Lens Opacities Classification System III (LOCS III). The anterior lens capsules were collected during surgery through continuous curvilinear capsulorhexis, immediately preserved in 4% paraformaldehyde, and stored at 4°C.

Immunofluorescence

Lens capsules were washed with phosphate-buffered saline (PBS) and permeabilized with 0.1% Triton X-100 in PBS for 20 minutes at room temperature. Subsequently, the tissue was blocked with 1% bovine serum albumin (BSA) with 0.1% Triton X-100 in PBS for 1 hour at room temperature. Capsules were then incubated overnight at 4 °C with the following primary antibodies: Kir4.1 (AB240876, Abcam), TRPV4 (PAS-114478, Invitrogen), and LRRC8A (AB254389, Abcam), each diluted 1:300 in PBS with 1% BSA. Afterward, the capsules were washed three times with PBS and incubated with the secondary antibody Alexa Fluor 488 Goat anti-Rabbit (AB150081, Abcam), diluted 1:2000 in PBS with 1% BSA, for 2 hours at room temperature, protected from light. Next, the capsules were washed twice with PBS, and nuclei were stained with DAPI (1 μg/mL) for 15 minutes at room temperature, also protected from light. Finally, capsules were washed twice with PBS and mounted on glass slides using 79% glycerol-PBS. Samples were visualized and photographed using a BioTek Cytation 5 Cell Imaging Multimode Reader. Image analysis and quantification of fluorescence intensity per cell were performed using the BioTek Gen5 software (Gen5^{TM}, version 3.17, BioTek Instruments, Inc., Vermont, USA).

Data Presentation and Statistics

Sample size justification. In the absence of prior estimates for the expression levels of the target proteins, the sample size was determined assuming a two-tailed, two-sample comparison of means ($\alpha = 0.05$, power = 0.80). Detecting a large effect (Cohen's $d = 0.8$) would require approximately 26 patients per group. To ensure adequate power to detect large-to-moderate effects while maintaining study feasibility, we initially planned to recruit 30 patients per group. However, patient sample availability depended directly on the number of surgeries performed at the hospital, which ultimately reduced the sample size to 25 patients per group. Analyses of specific protein subsets by immunofluorescence were considered exploratory.

An ordinal logistic regression model was employed to evaluate the association between clinical condition (diabetes group versus control group) and the severity of lens opacities. The dependent variables included four types of lens opacities: Nuclear Color (NC), ranked from NC1 to NC6; Nuclear Opalescence (NO), ranked from NO1 to NO6; Cortical Cataract (C), from C1 to C5; and Posterior Subcapsular Cataract (P), from P1 to P5. Assessments followed the standardized criteria of the Lens Opacities Classification System III (LOCS III). The primary independent variable was clinical condition (diabetes vs control), with biological sex (male vs female) included as a stratification factor. To accurately reflect data distribution, weighting was applied using the observed frequencies for each combination of clinical group and severity level, utilizing the model's weighting option. The proportional odds assumption was presumed to hold and confirmed with the test of parallel lines. A logit link function was used, and regression coefficients (β) were estimated along with their standard errors (SE), Wald statistics, p-values, and 95% confidence intervals (95% CI). Odds ratios were calculated by exponentiating the estimated coefficients. Statistical analyses were conducted with IBM SPSS Statistics (IBM, version 27, Chicago, USA). A p-value < 0.05 was considered statistically significant. The frequency percentages within the cataract classification were graphed.

Fluorescence intensity was quantified in three independent experiments across 600 cells per field for each protein. Immunofluorescence data are shown as the mean ± SEM and analyzed using GraphPad Prism 8 (GraphPad Software, version 8.0.2, La Jolla, USA). The normal distribution of data was confirmed with the Shapiro–Wilk test, and Levene's test was used to assess variance homogeneity among groups. Statistical significance was determined with one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. A p-value of ≤ 0.05 was considered statistically significant between the control and experimental groups. Details of each experiment are provided in the corresponding figure legends.

Results

Demographic and Clinical Characteristics

The demographic and clinical features of the study population are summarized in Table 1. A total of 50 patients were included, with 24 from the T2DM group and 26 from the control group (without a diagnosis of diabetes). The sample consisted of older adults, with a mean age of 70.9 ± 9.7 years. In the diabetes group, the average duration of the disease was 12.7 ± 9.05 years. Additionally, diabetic participants exhibited elevated fasting blood glucose and glycated hemoglobin (HbA1c) levels, consistent with their clinical diagnosis (Table 1). These measurements were not taken in the control group. The primary comorbidity identified in both diabetic and non-diabetic patients was arterial hypertension. In the non-diabetic group, the predominant concomitant pharmacological treatment consisted of antihypertensive drugs. Conversely, diabetic patients primarily received oral antidiabetic agents alongside antihypertensives. A small proportion of the study population exhibited risk factors associated with cataract development, such as a history of smoking or corticosteroid use (Table 1).

Table 1 Demographic, Anthropometric, Glycemic, and Pharmacological Profiles of T2DM and Non-Diabetic Patients

	Diabetes	**Control**
n	24	26
Age	68.5 ± 8.7	72.9 ± 10.3
Gender ratio: F/M	15 F / 9 M	17 F / 9 M
BMI	26.8 ± 1.0 (n=10)	30.3 ± 3.0 (n=13)
Glycemic control parameters		
Fasting glucose (mg/dL)	128.1 ± 34.3	–
HbA1c %	7.7 ± 1.6	–
Diabetes duration (years)	12.7 ± 9.0	–
Comorbidities and risk factors		
Smoking history	4 (0 F / 4 M)	5 (3 F/ 2 M)
Hypertension	15 (11 F / 4 M)	11 (6 F / 5 M)
Dyslipidemia	1 (1 F / 0 M)	8 (3 F / 5 M)
Hypothyroidism	4 (4 F / 0 M)	3 (2 F / 1 M)
Chronic kidney disease	1 (1 F / 0 M)	3 (1 F / 2 M)
Concomitant medications		
Corticosteroid therapy	1 (1 F / 0 M)	0
Oral antidiabetic agents	19 (12 F / 7 M)	1 (0 F / 1 M)
Insulin	9 (5 F / 4 M)	0
Antihypertensive agents	14 (7 F / 7 M)	8 (4 F / 4 M)
Thyroid hormone replacement therapy	4 (4 F / 0 M)	3 (2 F / 1 M)

Notes: Demographic and clinical data of the T2DM and control populations. Concomitant medication was divided into the following groups: oral antidiabetic agents (metformin, glimepiride, dapagliflozin, and linagliptin); insulin (glargine, degludec, NPH); Antihypertensive agents (losartan, telmisartan, irbesartan, valsartan, hydrochlorothiazide, nifedipine, and spironolactone); and Thyroid hormone replacement therapy (levothyroxine). F=female, M=male. n= 50. The age, body mass index (BMI), and glycemic control parameters are presented as mean ± SD.

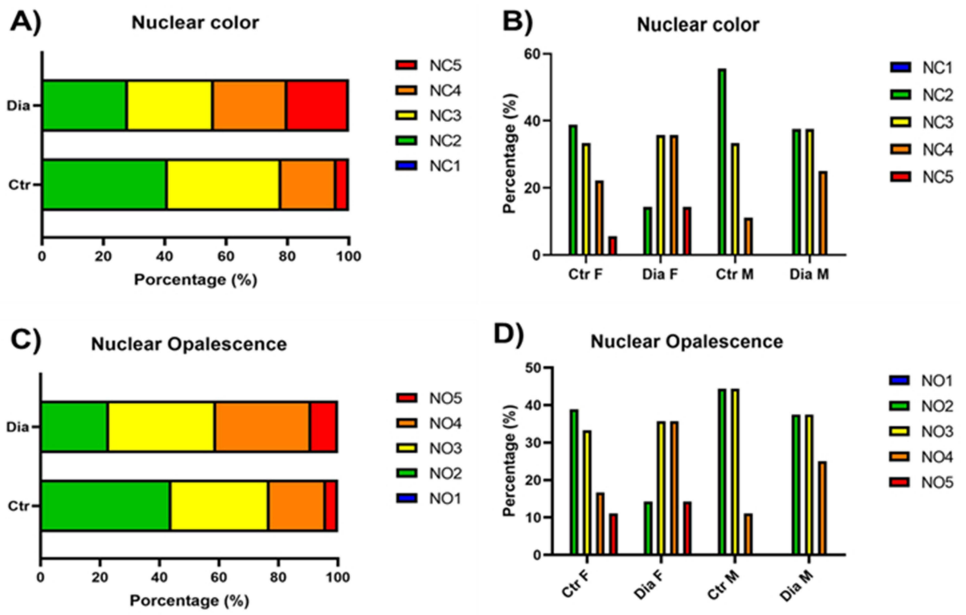

Figure 1 Cataract classification in control and diabetic groups was evaluated using the Lens Opacities Classification System III (LOCS III). Stacked bar charts display the percentage of patients according to severity grade for each cataract subtype: (**A** and **B**) Nuclear color (NC), and (**C** and **D**) Nuclear opalescence (NO). Each bar illustrates the percentage distribution of severity grades within the study groups. A detailed description of the grading scales is provided in the Results section. Ctr, control group (n=26); Dia, diabetes group (n=24); Ctr F, female control group (n=17); Ctr M, male control group (n=9); Dia F, female diabetes group (n=15); Dia M, male diabetes group (n=9).

Diabetes Increases the Severity of Cataract Characteristics

Cataract severity in patients was assessed using the Lens Opacities Classification System III (LOCS III), based on the following parameters: nuclear color (NC), nuclear opalescence (NO), cortical cataract (C), and posterior subcapsular Cataract (P).

The NC parameter indicates the level of coloration in the lens nucleus, with a scale from NC1 (light color) to NC6 (dark brown). In the control group, the highest percentage of patients was observed at NC2 (41%), followed by NC3 (37%), NC4 (18%), and NC5 (4%). In the diabetes group, the most significant proportions were found at NC2 and NC3 (28% each), followed by NC4 (20%), and NC5 (24%) (Figure 1A). When stratified by sex, women in the control group showed a higher percentage of cases in more severe grades (NC3-NC5) compared to men in the same group (Figure 1B). Similarly, in the diabetes groups, both men and women exhibited a higher percentage of patients in more severe grades, with this trend being more evident in diabetic women (Figure 1B).

These descriptive findings were supported by ordinal logistic regression analysis (Table 2). In the model comparing diabetic versus control participants regardless of sex, the presence of diabetes was related to increased severity of nuclear color, although the effect did not reach conventional significance ($\beta = 0.878$; 95% CI: −0.166 to 1.921; $p = 0.099$). However,

Table 2 Ordinal Logistic Regression Analysis of Lens Opacity Across Clinical Groups in Cataract Patients

Parameter	Model χ^2	*P*-value (Model)	Coefficient (β)	95% CI for β	*P*-value (β)
Nuclear Color (NC)	2.742	0.098	0.878	[−0.166, 1.921]	0.099
Nuclear Opalescence (NO)	3.160	0.075	0.946	[−0.111, 2.003]	0.079
Cortical Cataract (C)	10.117	0.001*	1.714*	[0.621, 2.807]	0.002
Posterior Subcapsular (P)	0.079	0.779	0.143	[−0.854, 1.141]	0.778

Notes: Ordinal logistic regression models (PLUM procedure, logit link function) were used to assess the association between clinical group (diabetes vs control) and the severity of each lens opacity subtype, as classified by the LOCS III system: nuclear color (NC), nuclear opalescence (NO), cortical cataract (C), and posterior subcapsular cataract (P). The control group was used as the reference category. The β coefficients represent the log-odds of exhibiting a higher severity grade associated with the diabetic condition. The χ^2 and p-values indicate model fit with predictors compared to the null model (without predictors). Statistically significant associations ($p < 0.05$) are indicated with an asterisk (*). Ctr, control group (n=26); Dia, diabetes group (n=24).

https://doi.org/10.2147/OPTH.S557358

when stratified by sex, diabetic females (Dia F) exhibited a significantly increased nuclear color severity compared to male controls (Ctr M) (β = 1.647; 95% CI: 0.049–3.245; $p = 0.043$), suggesting a sex-specific susceptibility (Table 3).

The NO parameter evaluates the diffuse or hazy opacity level in the lens nucleus, using a scale from NO1 (minimal opalescence) to NO6 (significant opacity). In the control group, the majority of patients were at NO2 (44%), followed by NO3 (33%), NO4 (19%), and NO5 (4%). In the T2DM group, NO3 was the most common grade (36%), then NO4 (32%), NO2 (23%), and NO5 (9%) (Figure 1C). When stratified by sex, both the female control group and the female diabetes group showed a higher percentage of advanced opalescence (NO3–NO5) compared to their male counterparts (Figure 1D).

Regression analysis revealed a trend toward greater nuclear opalescence in the diabetes group (β = 0.946; 95% CI: −0.111 to 2.003; $p = 0.079$) (Table 2). When analyzed by sex, diabetic women again exhibited significantly higher severity (β = 1.967; 95% CI: 0.311–3.622; $p = 0.020$), consistent with the descriptive data and indicating a meaningful increase in nuclear opacity within this subgroup (Table 3).

The cortical cataract (C) parameter assesses the presence of opacities in the lens cortex, using a scale from C0 (no involvement) to C5 (extensive involvement). In the control group, the majority of patients were classified as C0 (52%), followed by C2 (22%), C1 (11%), C3 (7%), and C5 (7%). By contrast, the patients with T2DM, the highest proportion was at C4 (27%), then C3 (23%), C1 (18%), C0 (14%), C2 (9%), and C5 (9%) (Figure 2A). Sex stratification revealed that the female diabetes group had a higher proportion of advanced cortical opacities (C3–C5) compared to other groups (Figure 2B).

Statistical analysis revealed a significant link between diabetes and increased cortical cataract severity in the overall model (β = 1.714; 95% CI: 0.621–2.807; $p = 0.002$) (Table 2). However, when broken down by sex, the association was no longer statistically significant, indicating that the effect seen in the unstratified analysis might be influenced by specific subgroups or sex-related factors (Table 3).

The grading scale for posterior subcapsular cataract (P) assesses opacity in the posterior region of the lens. This classification ranges from P0 (absent) to P5 (dense opacity). In the control group, the highest proportion of patients was at P0 (30%), followed by P3 (26%), P1 (22%), P5 (15%), and P2 (7%). In the diabetes group, the distribution was more heterogeneous: P0 (27%), P1 (23%), P4 (18%), P5 (14%), P2 (14%), and P3 (5%) (Figure 2C). Sex stratification revealed that the female control, female diabetes, and male control subgroups had a higher proportion of patients with more severe posterior subcapsular opacity (P4–P5) (Figure 2D).

Table 3 Sex-Specific Ordinal Logistic Regression of Cataract Severity Subtypes in Diabetic and Non-Diabetic Patients

Parameter	Model χ^2	*P*-value (Model)	Group	Coefficient (β)	95% CI for β	*P*-value (β)
Nuclear Color (NC)	5.176	0.159	Ctr-Fem	0.483	[–1.015, 1.981]	0.527
			Dia-Fem*	1.647*	[0.049, 3.245]*	0.043*
			Dia-Mal	0.482	[–1.289, 2.253]	0.594
Nuclear Opalescence (NO)	6.504	0.090	Ctr-Fem	0.817	[–0.722, 2.357]	0.298
			Dia-Fem*	1.967*	[0.311, 3.622]*	0.020*
			Dia-Mal	0.750	[–1.059, 2.560]	0.416
Cortical Cataract (C)	5.180	0.159	Ctr-Fem	–0.698	[–2.180, 0.783]	0.356
			Dia-Fem	0.778	[–0.729, 2.286]	0.312
			Dia-Mal	0.225	[–1.482, 1.933]	0.796
Posterior Subcapsular (P)	1.059	0.787	Ctr-Fem	–0.280	[–1.699, 1.139]	0.699
			Dia-Fem	0.235	[–1.244, 1.715]	0.755
			Dia-Mal	–0.489	[–2.190, 1.211]	0.573

Notes: Results of the ordinal logistic regression models (PLUM procedure, logit link function) were applied to evaluate the association between clinical groups stratified by sex and the severity of lens opacities. The analyzed groups were: female controls (Ctr-Fem), females with diabetes (Dia-Fem), and males with diabetes (Dia-Mal), using male controls (Ctr-Mal) as the reference group. The dependent variables correspond to the cataract subtypes classified according to the LOCS III system: nuclear color (NC), nuclear opalescence (NO), cortical cataract (C), and posterior subcapsular cataract (P). The β coefficient represents the log-odds of exhibiting a higher severity grade relative to the reference group. The χ^2 and p-values indicate the fit of the full model compared to the null model (without predictors). Statistically significant associations ($p < 0.05$) are marked with an asterisk (*). Ctr F, female control group (n=17); Ctr M, male control group (n=9); Dia F, female diabetes group (n=15); Dia M, male diabetes group (n=9).

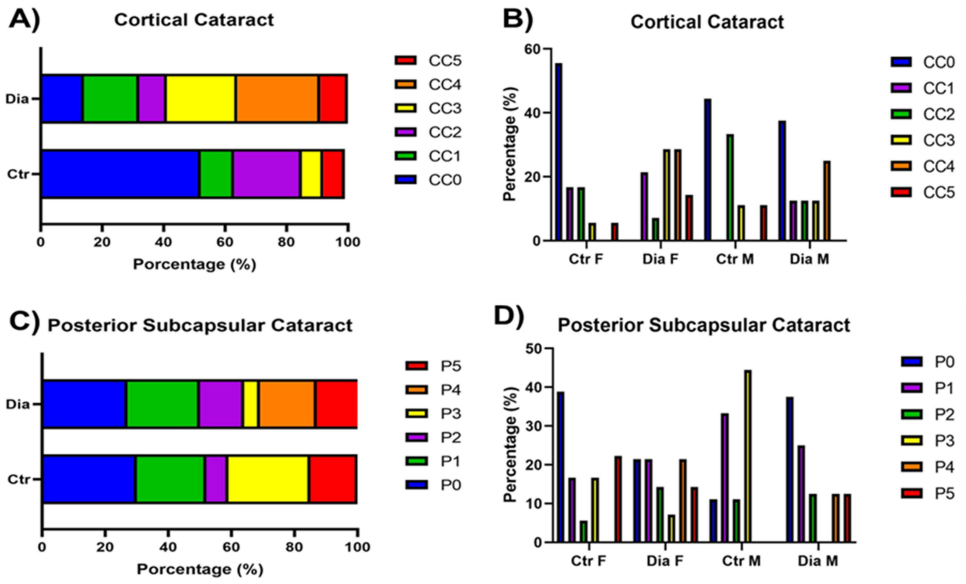

Figure 2 Cataract classification in control and diabetic groups was evaluated using the Lens Opacities Classification System III (LOCS III). Stacked bar charts display the percentage of patients according to severity grade for each cataract subtype: (**A** and **B**) Cortical cataract (C), and (**C** and **D**) Posterior subcapsular cataract (P). Each bar illustrates the percentage distribution of severity grades within the study groups. A detailed description of the grading scales is provided in the Results section. Ctr, control group (n=26); Dia, diabetes group (n=24); Ctr F, female control group (n=17); Ctr M, male control group (n=9); Dia F, female diabetes group (n=15); Dia M, male diabetes group (n=9).

Despite these descriptive trends, no significant association was found between diabetes and P severity (β = 0.143; 95% CI: −0.854 to 1.141; $p = 0.778$) (Table 2), even when stratified by sex. Additionally, in the sex-specific model, the assumption of proportional odds was violated ($p < 0.01$), which limits the interpretability of the ordinal model for this variable (Table 3).

Diabetes Alters the Levels of Osmotic Proteins in the Lens Capsules

Considering the osmotic stress caused by the increase of sorbitol in the lens of T2DM patients, the expression of the ion channels Kir4.1, TRPV4, and LRRC8A involved in osmotic regulation was examined in lens capsules by immunofluorescence.

Kir4.1 is a channel protein that regulates K+ ion transport, actively contributing to the maintenance of cellular osmotic pressure and water flux[20,21] Immunofluorescence analysis showed cytoplasmic labeling (Figure 3A). Quantification of the average fluorescence intensity per cell revealed a heterogeneous expression pattern among capsule cells (Figure 3B) and a significant increase in the mean fluorescence intensity observed in the T2DM group (Figure 3C).

TRPV4 is a nonselective cation channel primarily permeable to Ca^2+ and involved in detecting osmotic and mechanical changes.[22] Positive TRPV4 labeling was observed, showing a cytoplasmic distribution; some cells exhibited higher signal intensity in both the control and diabetes groups (Figure 4A). Fluorescence analysis demonstrated a heterogeneous signal pattern (Figure 4B) and a significant decrease in average intensity in the diabetes group (Figure 4C).

LRRC8 proteins, especially LRRC8A, are essential for cellular osmoregulation, functioning as key subunits of volume-regulated anion channels (VRACs).[23,24] These channels activate in response to cell swelling caused by osmotic stress, allowing ions and water to efflux to restore cell volume.[22,23] Positive labeling for LRRC8A was observed with a cytoplasmic distribution (Figure 5A). Fluorescence intensity analysis per cell revealed, like Kir4.1 and TRPV4 channels, a heterogeneous expression pattern (Figure 5B) and a notable decrease in average intensity in the diabetes group (Figure 5C).

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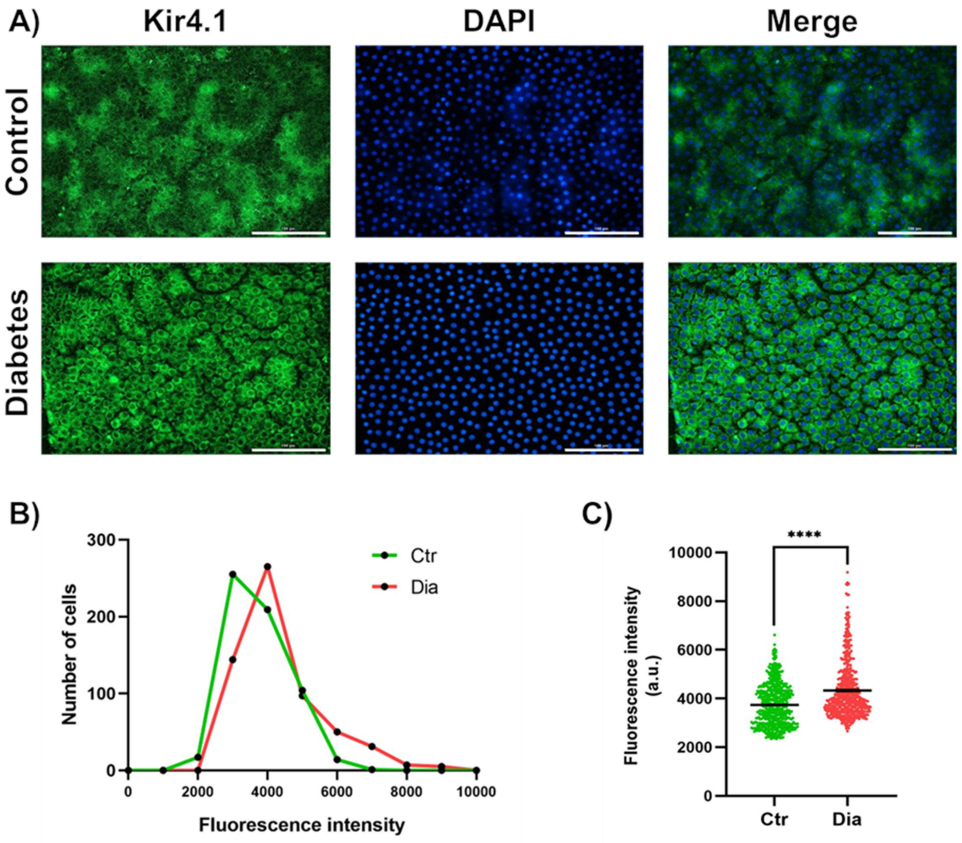

Figure 3 The diabetes condition increases Kir4.1 expression in lens capsules. (**A**) Immunofluorescence of Kir4.1 in lens capsules from control and diabetic patients. Kir4.1 labeling appears green, with nuclear staining using DAPI shown in blue. (**B**) Histogram illustrating the distribution of Kir4.1 fluorescence intensity per cell. (**C**) Comparison of mean fluorescence intensity per cell. Values represent fluorescence intensity per cell; the black bar indicates the mean ± standard error of the mean (SEM). For each patient, 600 cells were analyzed, resulting in 1800 quantified cells per group (diabetic and non-diabetic). ****$p < 0.0001$ compared to the control group (Ctr). The scale bar represents 100 μm.

Discussion

Cataracts remain a leading cause of reversible blindness worldwide, and their accelerated development in individuals with type 2 diabetes mellitus (T2DM) has long been recognized but is still not fully understood.[4] This study offers exploratory insights into molecular alterations in lens physiology associated with diabetes by combining clinical cataract grading with assessing osmoregulatory protein expression in human anterior lens capsules. Our findings reinforce the association between diabetes and greater cataract severity, and also identify specific molecular alterations in the expression of proteins linked to the osmotic stress response, which may be involved in the progression of this pathology.

Clinical Implications of Diabetes on Cataract Severity

Several clinical studies have reported that cataract formation occurs more frequently and at an earlier age in T2DM patients compared to the non-diabetic population.[4,7,13,14] The LOCS III-based grading showed that patients with T2DM had a higher frequency and severity of nuclear color (NC), and nuclear opalescence (NO) cataract subtypes, consistent with previous epidemiological data.[25,26] Although cataract severity was notably higher in the diabetic group overall, the sex-specific analysis indicated that diabetic women (Dia F) were especially vulnerable to more advanced grades of NC and NO, which are consistent with other epidemiological studies.[26,27] These subtypes affect color perception and night vision, critical for quality of life in aging populations.

While posterior subcapsular cataracts (P) have traditionally been linked to diabetes, this is due to the high sorbitol concentration in the posterior region of the lens capsule.[28] Our results did not find a significant connection between T2DM and this subtype, either overall or when analyzed by sex. This result may reflect a more complex pathophysiological interaction or

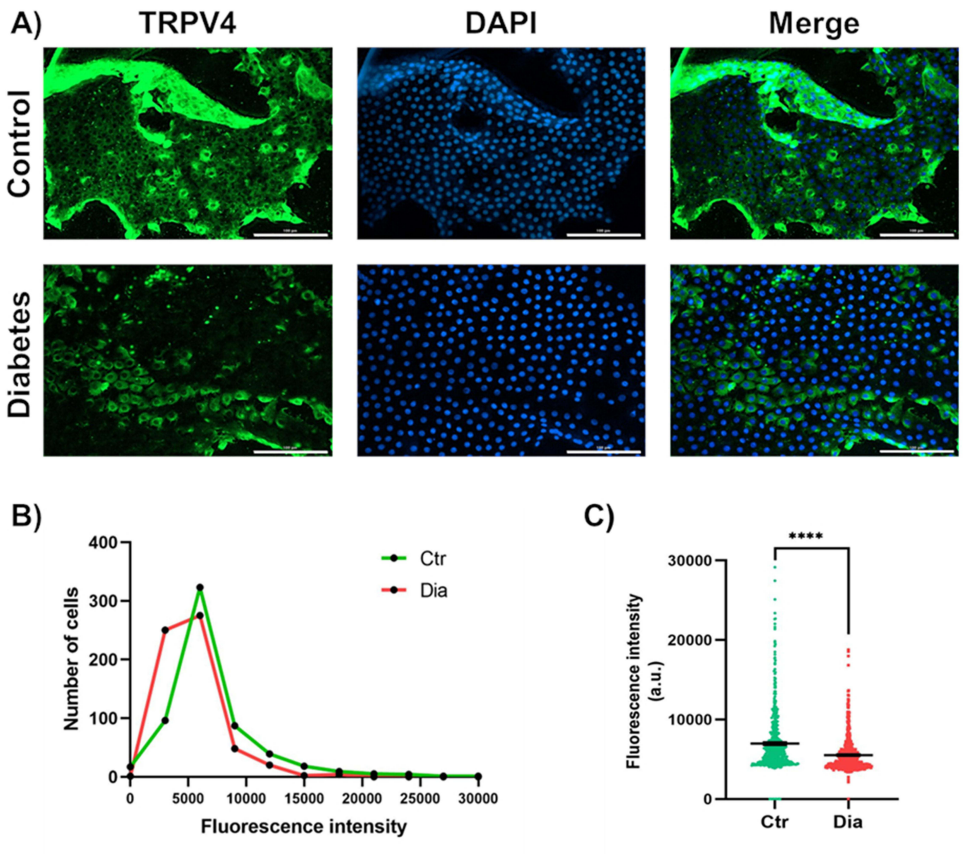

Figure 4 Diabetes decreases the expression of TRPV4 in lens capsules. (**A**) Immunofluorescence of TRPV4 in lens capsules from control and diabetic patients. TRPV4 labeling appears green, and nuclear staining with DAPI is blue. (**B**) Histogram showing the frequency distribution of TRPV4 fluorescence intensity per cell. (**C**) Comparison of the average fluorescence intensity per cell. Values indicate fluorescence intensity per cell; the black bar shows the mean ± standard error of the mean (SEM). For each patient, 600 cells were analyzed, resulting in 1800 quantified cells per group (diabetic and non-diabetic). ****$p < 0.0001$ compared to the control group (Ctr). The scale bar represents 100 μm.

variability within the diabetic population, including differences in glycemic control, disease duration, and comorbidities not fully captured in this cohort.[14,26,29] Although HbA1c levels, disease duration, and associated comorbidities were recorded in this study, the heterogeneity of the values and the lack of predefined stratification criteria limited our ability to perform robust subgroup analyses. Future studies with stratified designs and larger sample sizes may help clarify these relationships.

Most cataract cases develop as part of the natural aging process, during which lens fibers and proteins undergo breakdown and aggregation, ultimately leading to loss of transparency.[10,30] In the present study, participants were older adults, with a mean age of 70.9 ± 9.7 years old. A proportion of individuals in the control group exhibited high severity grades based on the LOCS III classification, which is directly associated with age-related degenerative changes.

Osmotic Stress Dysregulation as a Mechanistic Link

The lens is an avascular and metabolically specialized tissue highly susceptible to osmotic imbalance. In T2DM, chronic hyperglycemia leads to glucose diffusing into the lens, which is converted to sorbitol via aldose reductase.[15] The sorbitol accumulation disrupts osmotic gradients, resulting in water influx, fiber swelling, and light scattering.[17,31] However, the lens has osmoregulatory mechanisms to counteract these effects. One crucial response involves the coordinated activity of TRPV4, LRRC8A, and Kir4.1 channels, which work together to facilitate regulatory volume decrease (RVD),[17,23,32] an essential process that prevents cellular swelling and maintains transparency.

In our study, immunofluorescence showed a significant reduction in TRPV4 and LRRC8A expression in diabetic lens capsules and a notable increase in Kir4.1 expression. TRPV4, a mechanosensitive and osmosensitive Ca^{2}+-permeable channel, is crucial for sensing hypotonic stress and initiating downstream osmolyte efflux.[32] LRRC8A, a key part of

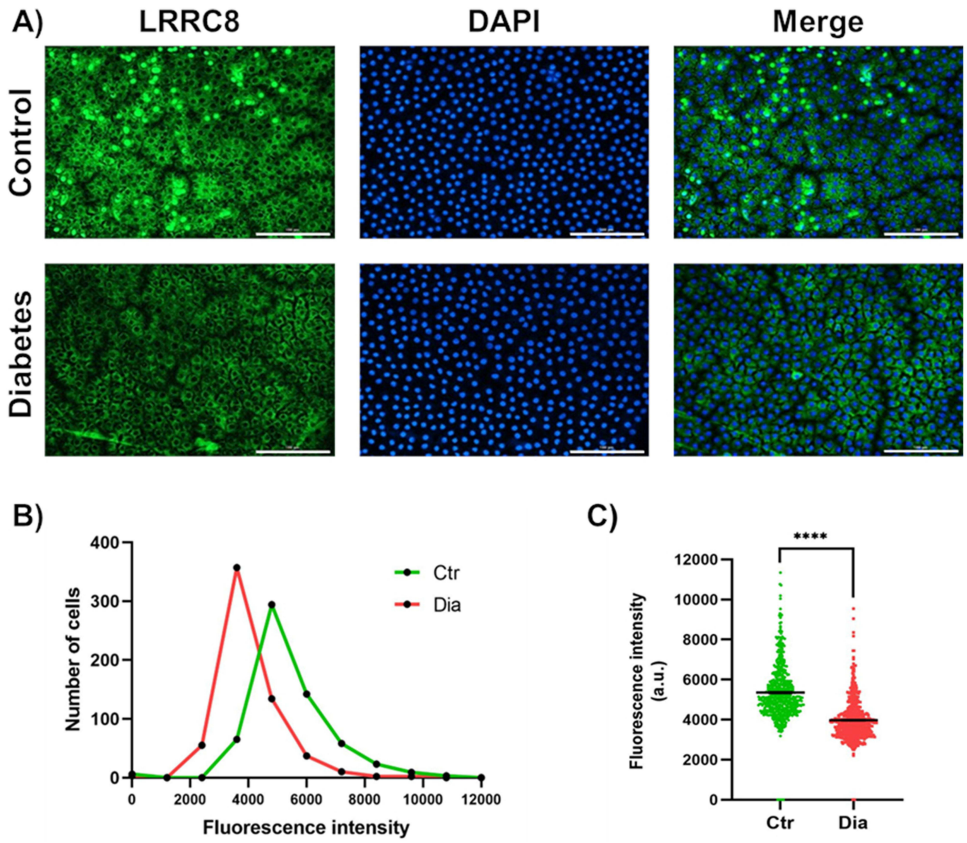

Figure 5 Diabetes reduces LRRC8 expression in lens capsules. (**A**) Immunofluorescence images of TRPV4 in lens capsules from control and diabetic patients. LRRC8 is shown in green, and nuclear staining with DAPI is shown in blue. (**B**) Histogram displaying the distribution of LRRC8 fluorescence intensity per cell. (**C**) Comparison of the average fluorescence intensity per cell. Values show fluorescence intensity per cell; the black bar represents the mean ± standard error of the mean (SEM). For each patient, 600 cells were analyzed, resulting in 1800 quantified cells per group (diabetic and non-diabetic). ****$p < 0.0001$ compared to the control group (Ctr). The scale bar indicates 100 μm.

volume-regulated anion channels (VRACs), is vital for Cl^- and osmolyte efflux during RVD.[23,33] Its decreased levels in diabetic lenses suggest impaired activation of this protective response. Likewise, reducing LRRC8A could further impair the lens's ability to recover from osmotic swelling.

In contrast, Kir4.1, an inwardly rectifying K^+ channel, was upregulated. While this may serve as a compensatory mechanism to facilitate K+ efflux and partially support RVD, it is unlikely to compensate for the absence of upstream activation via TRPV4 or the loss of anion channel function via LRRC8A. Together, these data suggest a maladaptive remodeling of the osmotic regulatory network in diabetic lenses, where compensatory increases in some channels are insufficient to restore homeostasis because primary sensors and effectors are downregulated. This dysregulation probably contributes to chronic osmotic stress, cytoskeletal disruption, and the ongoing loss of transparency. However, it is important to note that protein expression alone does not confirm functional activity. The lack of electrophysiological or osmotic flux assays limits our ability to infer channel functionality or causality.

Sex Differences and Translational Relevance

An important aspect of this study is identifying sex-specific differences in cataract severity among T2DM patients. Diabetic females exhibited significantly higher grades of NC and NO compared to their male counterparts and controls. These findings are consistent with previous reports indicating that women with diabetes are more likely to develop and experience progression of cataracts, especially nuclear types.[29,34] Although this study did not examine hormonal profiles, estrogen and other sex hormones influence lens metabolism, redox balance, and possibly the function or expression of osmotic channels. This hormonal modulation may worsen the molecular deficits seen in diabetic lenses, increasing women's vulnerability to osmotic imbalance and fiber damage.[23,33,35]

From a clinical perspective, these findings emphasize the importance of earlier and more careful ophthalmologic screening for diabetic women, possibly using sex-specific risk models for cataract development. Understanding the molecular mechanisms also provides opportunities for targeted drug treatments. For instance, modulating TRPV4 activity has shown promise in preclinical models of volume dysregulation and might serve as a therapeutic target to restore osmotic balance in the diabetic lens.

Limitations and Future Directions

Although this study integrates clinical and molecular analyses using human anterior lens capsules, several limitations must be acknowledged. The non-randomized and unmatched design limited control over potential clinically relevant factors such as age, sex, and cataract severity. Although analyses were stratified by sex and weighted according to observed frequencies to minimize bias, residual confounding factors cannot be excluded. Including sex as a secondary variable allowed for partial adjustment; however, future studies employing randomized or matched designs should be implemented to validate these findings with greater methodological robustness.

The sample size, although sufficient to detect significant differences in protein expression, may restrict the generalizability of the results, particularly regarding sex-specific trends. The subgroup of diabetic women (n = 15) was notably small, limiting the ability to draw definitive conclusions or perform robust multivariate analyses. Larger cohorts designed to explore sex-based differences and clinical covariates are essential to confirm these exploratory observations.

Additional clinical factors, such as smoking history, corticosteroid use, comorbidities, and concomitant medications, were collected but showed very low prevalence in the study population. This situation limited the feasibility of statistical adjustment or subgroup analysis. Although these data were descriptively reported, future studies should incorporate systematic collection and stratification of such risk factors to fully understand their potential influence on molecular outcomes fully.

The analysis focused exclusively on protein expression and did not include functional assays to assess channel activity, osmotic flux, or upstream regulatory mechanisms such as oxidative stress or inflammatory signaling. As a result, the mechanistic interpretation of the findings remains limited. This limitation suggests that future studies should incorporate ex vivo functional analyses or animal models to validate the roles of TRPV4, LRRC8A, and Kir4.1 in osmotic regulation of the diabetic lens. Moreover, the anterior lens capsule was used due to its accessibility during cataract surgery via continuous capsulorhexis, which allows for the collection of viable samples without interfering with the clinical procedure. This region may not fully represent the molecular changes in the cortical or nuclear lens fibers, where diabetic cataracts are more prominently manifested. Nevertheless, the lens capsule plays an active role in transporting water and metabolites to the cortical and nuclear fibers; therefore, an imbalance in its osmotic regulation could directly impact the internal homeostasis of the lens.

Importantly, the study did not stratify patients by HbA1c levels or duration of diabetes, despite collecting these variables. The heterogeneity of values and absence of predefined clinical cutoffs precluded formal subgroup analysis. Future research should incorporate stratified designs and larger sample sizes to explore potential correlations between metabolic control and osmotic channels expression.

Finally, while the study provides valuable insights into the differential expression of osmotic channels in diabetic cataracts, the absence of functional validation limits causal inference. The observed associations may reflect cataract severity rather than diabetes per se. Longitudinal studies with follow-up data, functional assays, and mechanistic exploration—such as the role of sex hormones in channel regulation—could clarify whether restoring TRPV4, LRRC8A, or Kir4.1 expression mitigates osmotic damage and contributes to sex-specific vulnerability. Such approaches may inform targeted prevention strategies and enhance understanding of lens pathophysiology in diabetes.

Conclusion

This study provides exploratory evidence that the regulation of osmotic stress is significantly altered in the lens of individuals with type 2 diabetes mellitus, which may contribute to the early development and increased severity of cataracts. The observed decrease in TRPV4 and LRRC8A, together with the upregulation of Kir4.1 expression, suggests a dysregulation of the lens's osmoregulatory capacity. Recognizing and addressing these changes could lead to developing novel diagnostic markers and therapeutic options for diabetic cataracts.

Abbreviations

T1DM, Type 1 diabetes mellitus; T2DM, Type 2 diabetes mellitus; DM, Diabetes mellitus; RVD, Regulatory Volume Decrease; RVI, Regulatory Volume Increase; PBS, Phosphate-buffered saline; BSA, Bovine serum albumin; TRPV4, Transient Receptor Potential Cation Channel Subfamily V Member 4; LRRC8, Leucine-rich repeat-containing protein 8A; Kir4.1, Inwardly rectifying potassium channel 4.1; VRACs, Volume-regulated anion channels.

Data Sharing Statement

The datasets generated and analyzed during this study are available from the corresponding author upon reasonable request.

Ethics Approval and Informed Consent

The Institutional Review Board (IRB) and the Ethics and Research Committee of the Association to Prevent Blindness in Mexico, under number INV-22-03, approved this study (protocol: LOP.PTR.22-01 Version 2, 13 de octubre del 2022). All participants gave written informed consent.

Consent for Publication

All participants provided consent for anonymized data and images to be published.

Acknowledgments

We thank the staff at Asociación para Evitar la Ceguera en México, I.A.P., Hospital "Dr. Luis Sánchez Bulnes", Karla Tovar Hernández, and Nadia Flores Huerta for their assistance during surgery and sample collection.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

The authors declare that they received financial support for this article's research, authorship, and/or publication. This work was funded by UNAM (PAPIIT IN221023), Facultad de Medicina UNAM, Asignación presupuestal Investigadores 2024, and APEC (LOP.PTR.22-01). Dr. Jesus Silvestre Albert Garay holds a postdoctoral research fellowship funded by SECIHTI through the Postdoctoral "Investigadores por México", SECIHTI (CVU 789988). Alan Emmanuel Medina Arellano is a PhD student in the Programa de Doctorado en Ciencias Biomédicas at UNAM (CONAHCYT fellowship 895582).

Disclosure

The authors state that they have no conflicting financial or non-financial interests.

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