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Comparison Between Irisin Levels in Aqueous Humor of Pseudoexfoliation and Non Pseudoexfoliation Cataract Patients

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Abstract

The study's objective was to assess and compare the concentration of irisin in the aqueous humor of patients with and without pseudoexfoliation cataracts. Two groups of 60 patients participated in the study. Group I consisted of 30 patients with cataracts and pseudoexfoliation, whereas Group II consisted of 30 patients with cataracts but without pseudoexfoliation. We conducted a comprehensive ophthalmological examination and collected aqueous humor throughout the surgery. We used an enzyme-linked immunosorbent assay (ELISA) with aqueous humor as the sample to determine the level of irisin. Group II had a considerably lower level of aqueous humor irisin (41.73± 11.59 ng/ml) compared to group I (56.20± 10.5 ng/ml, P = 0.001) when comparing the two groups. There was a negligible connection between visual acuity and the level of irisin in the aqueous humor (P = 0.55). Additionally, we observed a negligible connection between intraocular pressure and the irisin level in the aqueous humor (P = 0.16), as well as visual acuity (P = 0.55). The study reveals that cataract patients with pseudoexfoliation syndrome (PEXS) exhibited elevated levels of irisin in both their serum and aqueous humor in comparison to individuals without PEXS. This suggests a possible link between irisin and the development of PEXS-related cataracts.

Keywords: Irisin, Cataract, Pseudoexfoliation, Non Pseudoexfoliation.

Introduction

Irisin is a 112-amino acid hormone-like myokine discovered in 2012 (Bostrom et al 2012). It was discovered in presence of exercise-induced peroxisome proliferate-activated receptor gamma coactivator-1-alpha (PGC-1α) that plays an important role in energy regulation in each organ in the body and regulation of metabolic diseases as diabetes and obesity. The Irisin discovery can contribute to new effective therapeutic targets and therapeutic strategies of metabolic diseases or metabolism-associated health issues (Chen et al. 2016).

Irisin is a cleavage product of fibronectin type III domain-containing protein 5 (FNDC5) that converts white adipocytes to brown adipocytes (Fu et al. 2021). Irisin is present in various organs: brain, heart, liver, and skeletal muscle and interferes in controlling metabolism of glucose, lipid and energy homeostasis in skeletal muscle and adipose tissue (Aladag et al. 2023).

Multiple studies showed that irisin has anti-inflammatory, antioxidant and anti-apoptotic effects (Askari et al. 2018). Irisin crosses blood–brain barrier and its expression had been found in some sites of the central nervous system (Islam et al. 2017). Studies had shown that irisin could enhance the expression of brain-derived neurotrophic factor (BDNF) in various brain regions with subsequent beneficial effects on brain health and cognitive function (Pesce et al. 2021). In the central nervous system, irisin has neuroprotective effects, promotes neurogenesis, and modulates plasticity of neural synapses (Kim and Song, 2018).

Irisin has received little focus in ophthalmology investigations, as noted by Güler et al. in 2020. A study demonstrated that there is no significant variation in circulating irisin levels across different kinds of open-angle glaucoma and normal controls. According to Turgut et al. (2019), irisin could potentially have a role in the development of glaucoma and may operate as a protective hormone for the nervous system in this condition.

Researchers have found increased levels of Irisin in the aqueous humor in individuals with high myopia, opening a new avenue for investigating the relationship between physical activity and myopia. Furthermore, there is a direct correlation between irisin concentrations and axial length. However, further clarification is required to comprehend the precise mechanisms through which Irisin drives the progression of myopia (Wang et al., 2021).

Turgut et al. (2019) assessed the levels of irisin in the serum of individuals with chronic open-angle glaucoma. Despite the glaucoma groups appearing to have lower average irisin levels than the control group, there was no statistically significant disparity in serum irisin levels among the tested groups. The results suggest that the levels of irisin in the blood stay consistent in people with chronic open-angle glaucoma. Limited neurodegenerative mechanisms occurring in the optic nerve could account for the absence of substantial findings concerning serum irisin levels in this investigation. These findings indicate that irisin may have diverse effects on different tissues, including the blood and neurons. Conversely, the concentrations of irisin in samples collected from the vitreous or aqueous humor may vary between individuals with glaucoma and those without the condition. Furthermore, the concentrations in such samples may either drop or rise in comparison to the levels seen in blood serum.

In eyes with age-related macular degeneration (AMD) and retinal vein occlusion, the amount of irisin in the aqueous humor drops. Moreover, there is a documented correlation between elevated levels of irisin and augmented macular thickness, particularly in cases of retinal vein occlusion. This result necessitates additional inquiry into the potential association between irisin and the incidence of macular edema (Li et al., 2022).

According to a study by Ambreen et al. (2021), irisin has the capacity to reduce oxidative stress, indicating that it may operate as a preventive factor against age-related cataracts.

The association between serum irisin and diabetic retinopathy is controversial. Irisin level was not different between patients with non-proliferative diabetic retinopathy and patients without retinal diabetic changes **(El Haddad et al. 2019)**. On the other hand, **Hu et al. 2016** found that patients with proliferative diabetic retinopathy had significantly lower irisin in serum and vitreous compared with patients without diabetic retinopathy in Type 2 Diabetes Mellitus.

Not much research has been done on the link between irisin levels and getting cataracts or how its levels change in people with pseudoexfoliation, specifically in the aqueous humor. This study aims to shed light on this subject.

The study's objective was to assess and evaluate the concentration of irisin in the aqueous humor of individuals with pseudoexfoliation and non-pseudoexfoliation cataracts.

Material and Method

The study was cross-sectional and enrolled patients from the ophthalmology department of the Faculty of Medicine, Aswan University, between January 2023 and January 2024. The Scientific and Ethical Committees at the Faculty of Medicine (ASW.UNI./600/2/22), Aswan University, reviewed and approved the study. All participants provided informed written consent. The study included 60 patients in total, divided into two groups. Group I consisted of 30 patients with cataracts and pseudoexfoliation, whereas Group II consisted of 30 patients with cataracts without pseudoexfoliation

Inclusion criteria:

Patients with pseudoexfoliation and non pseudoxfoliation cataract aged between 50 and 70 years.

Exclusion criteria:

1- Patients with other eye diseases as age related macular degeneration (ARMD) and high myopia.
2- Patients with baseline Intra-ocular pressure greater than 21 mm Hg.
3- Patients with history of ocular surgery
4- Patients with history of diabetes mellitus and hypertension.

Each patient was assessed by:

1) Complete history taking including baseline information: patients' age, gender, any systemic diseases, any ocular diseases, any ocular medications and previous ocular surgeries.
2) Full ophthalmological examination including best corrected visual acuity, intraocular pressure by Goldman applanation tonometry, slit-lamp biomicroscopic examination.
3) Presence of pseudoexfoliation (PEX) was determined examining dilated pupil examination using slit-lamp showing white dandruff-like deposits on the anterior lens capsule and the pupillary margin, fundus examination using +90 D lens (Volk Optical Inc., Mentor, OH, USA).
4) Patients were divided into 2 groups:
   Group I: 30 Patients with cataract with pseudoexfoliation.
   Group II: 30 Patients with cataract without pseudoexfoliation.

- Aqueous humor samples 0.1 to 0.2 ml were aspirated from all patients at the time of cataract surgery through a limbal paracentesis via a 27-gauge cannula mounted on a tuberculin syringe , special attention was paid to avoid contamination of aqueous humor samples with blood.
- Phacoemulsification was done using (Faros, Oertli, Switzerland) phaco-vitrectomy machine. under local peribulbar anaesthesia by 1:1 mixture of Xylocaine 2% and Bupivicaine 0.5% .
- Collected aqueous humor and serum samples were analyzed using enzyme-linked immunosorbent assay (ELISA) kits to detect irisin concentrations.
- Assesment of Irisin levels in aqueous humor: Specimen Collection:

Aqueous Humor: was collected by sterile tube. Centrifuged at 2000-3000 RPM for 20 minutes. Collected the supernatant without sediment and stored at (-30 ℃) until assayed.

Assay Principle This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human Irisin antibody. Irisin present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human Irisin Antibody is added and binds to Irisin in the sample. Then Streptavidin-HRP was added and bounnd to the Biotinylated Irisin antibody. After incubation unbound Streptavidin-HRP was washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human Irisin. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Reagent Preparation

- All reagents was brought to room temperature before use. Standard Reconstitute the 120ul of the standard (1280ng/mL) with 120ul of standard diluent to generate a 640ng/mLstandard stock solution. Allowed the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepared duplicate standard points by serially diluting the standard stock solution (640ng/mL) 1:2 with standard diluent to produce 320ng/mL, 160ng/mL, 80ng/mL and 40ng/mL solutions. Any remaining solution should be frozen at (-20°C) and used within one month.

Dilution of standard solutions suggested are as follows:

640ng/mL	Standard No.5	120ul Original standard + 120ul Standard diluent
320ng/mL	Standard No.4	120ul Standard No.5 + 120ul Standard diluent
160ng/mL	Standard No.3	120ul Standard No.4 + 120ul Standard diluent
80ng/mL	Standard No.2	120ul Standard No.3 + 120ul Standard diluent
40ng/mL	Standard No.1	120ul Standard No.2 + 120ul Standard diluent

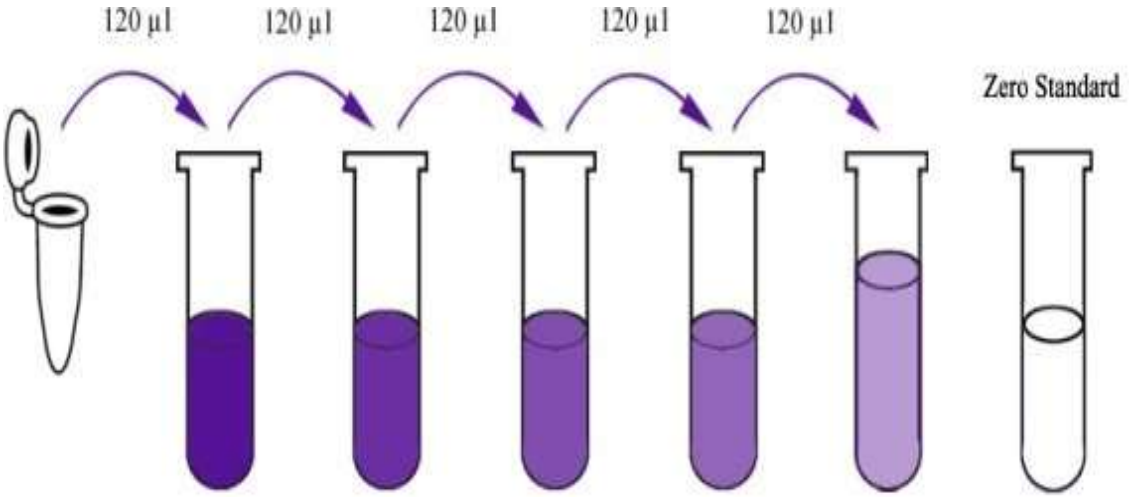

Fig.8 Dilution of standard solution procedure

Standard concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
1280ng/mL	640ng/mL	320ng/mL	160ng/mL	80ng/mL	40ng/mL

Washed Buffer Diluted 20ml of Wash Buffer Concentrate 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mixed gently until the crystals have completely dissolved.

Assay Procedure:

1. Prepared all reagents, standard solutions and samples as instructed. Brought all reagents to room temperature before use. The assay was performed at room temperature.
2. Determined the number of strips required for the assay. Inserted the strips in the frames for use. The unused strips should be stored at 2-8°C.
3. Added 50ul standard to standard well. Note: Didn't add antibody to standard well because the standard solution contains biotinylated antibody.
4. Added 40ul sample to sample wells and then added 10ul Human FNDC5 antibody to sample wells, then added 50ul streptavidin-HRP to sample wells and standard wells (Not blank control well). Mixed well. Covered the plate with a sealer. Incubated 60 minutes at 37°C.
5. Removed the sealer and washed the plate 5 times with wash buffer. Soak wells with 300ul wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirated or decant each well and washed 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material.
6. Added 50ul substrate solution A to each well and then add 50ul substrate solution B to each well. Incubated plate covered with a new sealer for 10 minutes at 37°C in the dark.
7. Added 50ul Stop Solution to each well, the blue color will change into yellow immediately.
8. Determined the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

Statistical analysis:

The statistical analysis was performed using SPSS version 25 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0.Armonk, NY: IBM Corp.).

Results

The study contained two groups, each consisting of 30 subjects: group I (patients with cataracts and pseudoexfoliation) and group II (patients with cataracts without pseudoexfoliation). Table 1 presents the demographic data of the subjects under investigation.

When comparing the levels of irisin in the aqueous humor between group I and group II, it was found that group II had a considerably lower level of irisin (41.73± 11.59 ng/ml) compared to group I (56.20± 10.5 ng/ml, P = 0.001) (see table 2, fig. We found a statistically negligible connection (P = 0.55) between the irisin level in the aqueous humor and visual acuity. In addition, there was a negligible association between intraocular pressure and the level of irisin in the aqueous humor (P = 0.16), as well as visual acuity (P = 0.55) (table 3).

Table (1): Demographic data of the studied groups

		Group I (n=30)	Group II (n=30)	P
Age (mean ±SD) years		60.1±6.3	60.27±7.2	**0.85****
Sex No (%)	Male	13 (43.3%)	14 (46.7%)	**0.83***
	Female	17 (56.7%)	16 (53.3%)	

Table (2): Comparison between Aqueous humor irisin levels between group I and group II

Group	Group I (N=30) Mean± SD	Group II (N=30) Mean± SD	P
Aqueous Humor irisin ng/ml	56.20± 10.5	41.73± 11.59	P=0.001 (ANOVA test)

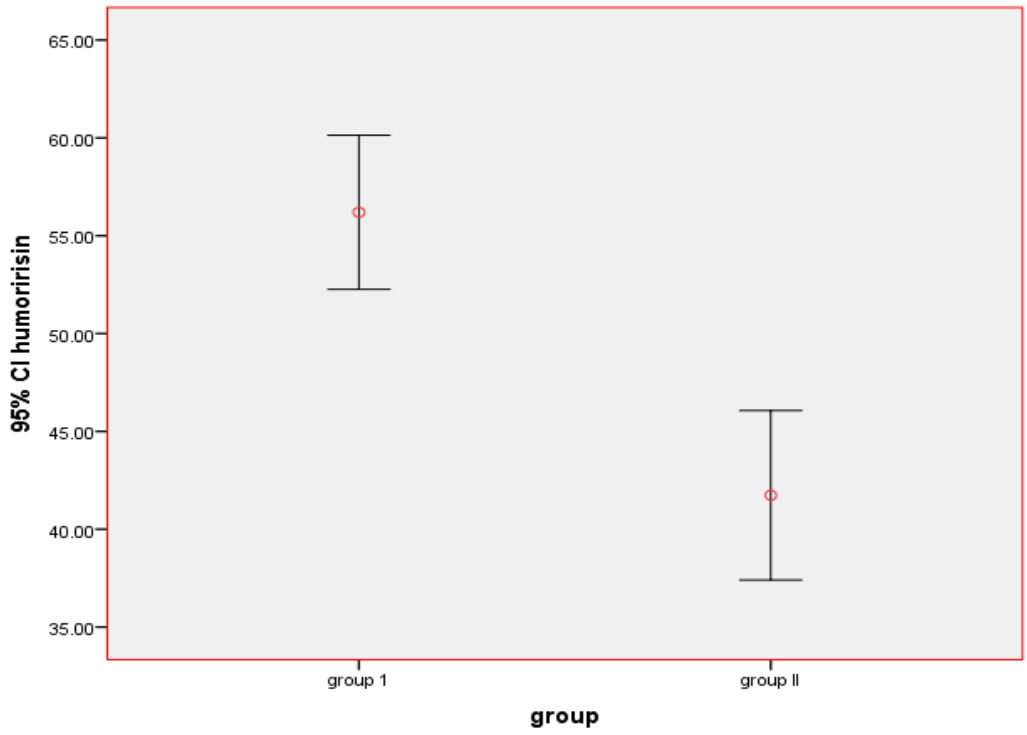

Figure (1): Comparison between aqueous humor irisin levels between group I and group II

Table (3): Correlation between visual acuity, intraocular pressure, serum irisin level, and humor irisin level in group I (patients with cataract with pseudoexfoliation)

Variable	Aqueous humor irisin	Visual acuity
Aqueous humor irisin		r = 0.11 P=0.55
Visual acuity	r= 0.11 P=0.55	
Intraocular pressure	r= -0.27 P=0.16	r= -0.11 P=0.55

Pearson Correlation, r (correlation coefficient)

Discussion

Cataract, identified as the leading cause of global blindness (Cicenilli et al., 2023), holds significant importance in clinical and biological research. Pseudoexfoliation (PEX) is an age-related systemic disease that mostly impacts the front structures of the eye. It is also the leading cause of secondary open-angle glaucoma (Schweitzer 2018). Research on PEX's pathophysiology and its accompanying systemic effects, as well as potential biomarkers, is critical.

The demographic data presented in this study provide useful insights into the characteristics of the participants in the three groups, helping to identify potential confounding factors and facilitating the interpretation of the study findings. When it comes to age distribution, The average age of participants in each group was roughly similar. Group I consisted of cataract patients with pseudoexfoliation, whereas Group II consisted of cataract patients without pseudoexfoliation. The mean age of both groups was approximately 60 years, as senile cataract is a disease that occurs with age. The study findings' homogeneity in age distribution helps to alleviate any potential age biases.

Our findings indicate that the levels of irisin in the aqueous humor were considerably lower in group II (41.73 ± 11.59 ng/ml) compared to group I (56.20 ± 10.5 ng/ml). We can rationalize the potential reasons for these increases associated with PEXS. The accumulation of atypical fibrillar substances in the eye, leading to diverse alterations in ocular tissues, distinguishes PEXS. Chronic inflammation and oxidative stress significantly impact the microenvironment of the eye, influencing PEXS. These processes have the potential to interfere with regular cellular functioning, such as the synthesis, secretion, and elimination of substances like irisin.

Furthermore, it is possible that the pathological processes linked to pseudoexfoliation syndrome (PEXS) may impact irisin, a myokine that plays a role in regulating metabolism and controlling inflammation. Disruptions in metabolic pathways, in addition to ongoing inflammation and oxidative stress, can impact ocular tissues' production and release of irisin. Because of this, changes in the amount of irisin in the eye fluid may show what else is wrong with people who have pseudoexfoliation syndrome (PEXS).

In their study, Güler et al. (2020) looked at aqueous humor samples from 30 people who only had cataracts and 31 people who had cataracts and pseudoexfoliation syndrome (PEX) but no glaucoma. They measured the levels of irisin, heat-shock protein 70, and periostin in these samples. The results showed that these proteins were approximately 1.5 times higher in patients with cataracts and PEX compared to the controls without PEX. The researchers found a correlation between this increase in protein levels and the presence of subclinical inflammatory processes and oxidative damage in PEX patients.

The findings in the aforementioned study align with our own results. In our study, people with both cataracts and pseudoexfoliation syndrome (PEXS) had about 1.35 times higher levels of irisin in their aqueous humor than cataract patients who did not have PEX.

Our findings, along with those from Güler et al. (2020), demonstrate a link between PEXS and a subclinical inflammatory process and oxidative stress, leading to an increase in irisin levels in the aqueous humor. Both investigations excluded participants with a prior medical history of diabetes mellitus, systemic hypertension, other eye-related disorders, or previous eye procedures. Higher levels of irisin, especially in certain forms of cataracts or in patients with pseudoexfoliation syndrome (PEX), may suggest a greater likelihood of disease progression or consequences. Medical professionals can use this data to closely observe patients and administer proactive treatments to alleviate further deterioration. Healthcare professionals can utilize irisin levels as a prediction tool to intervene at an early stage, which has the potential to enhance patient outcomes and optimize management techniques for cataracts and disorders such as pseudoexfoliation.

The study reveals that cataract patients with pseudoexfoliation syndrome (PEXS) exhibited elevated levels of irisin in aqueous humor as compared to individuals without PEXS. This suggests a possible link between irisin and the development of cataracts associated with PEXS.

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