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NOVEL ROLE OF MYOKINE IRISIN IN SENILE CATARACT

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ABSTRACT

Fibronectin type III domain-containing protein (FNDC) 5, the precursor of irisin, is encoded by the FNDC5 gene. Irisin is secreted from skeletal muscle and helps in metabolism by increasing energy level. Currently the change in irisin levels was estimated in aqueous humor of cataract patients compared to serum levels of cataract and normal subjects. The aqueous humor and blood samples were collected from senile cataract patients (N=50). Blood samples of normal age related subjects (N=50) were also collected. Irisin was estimated in aqueous humor and serum of cataract patients and normal controls through ELISA. Independent sample t-test was applied to compare the irisin concentration of aqueous humor and blood samples of senile cataract patients; and comparison of serum irisin levels between control and senile cataract patients. Probability level ($p < 0.05$) was considered significant. Irisin levels were reduced significantly ($p < 0.0001$) in aqueous humor of senile cataract patients compared to the serum levels. However, irisin levels in serum of control subjects was non-significantly ($p < 0.129$) different from the senile cataract patient serum. The study concludes that being an anti-inflammatory protein, reduced irisin levels may be a contributing factor for cataract pathology.

Keywords: senile cataract, irisin, serum, aqueous humor, Pakistan,

INTRODUCTION

Irisin, named for the Greek messenger goddess, was discovered in a screen looking for factors secreted by muscles in response to peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α) activation (Boström *et al.,* 2012). Fibronectin type III domain-containing protein 5 (FNDC5) is a glycosylated type I membrane protein. It contains an N-terminal signal peptide (amino acid (aa) 1-28), a FNIII domain (aa 33 – 124), a transmembrane domain (aa 150 – 170), and a cytoplasmic tail (aa 171 – 209). The secreted form of FNDC5 contains 112 amino acids (aa 29-140), named irisin, and is generated by proteolytic cleavage and is released into the circulation. The FNIII-like domain shows an unusual conformation with continuous inter-subunit beta-sheet dimer, which has not been previously described for any other FNIII protein. Irisin is 100% conserved from mouse to human and is highly conserved across mammals. Irisin has been crystallized and its structure has been dissolved (Schumacher *et al.,* 2013).

Irisin, a small protein containing the FNDC5 was initially discovered in a genomic search, with a focus on FNIII domains (Teufel *et al.,* 2002). Cleavage in the linking peptide releases soluble irisin into the extracellular matrix (Boström *et al.,* 2012). FNDC5 expressed in skeletal muscle, pericardium, heart and brain, was originally discovered as a receptor and shown to be critical for the differentiation of myoblasts and neurons (Hashemi *et al.,* 2013). Since its discovery, multiple studies have focused on the physiological role(s) of irisin or the FNDC5 ectodomain in metabolism (Dun *et al.,* 2013).

Certain well characterized beneficial effects of exercise in muscle, including white-to-brown fat conversion are stimulated by PGC-1α (Handschin and Spiegelman, 2008). Expression of FNDC5 in skeletal muscle was increased in mice with transgenically increased PGC-1α in skeletal muscle and reduced in mice with muscle-specific deletion of PGC-1α. The strong correlation between mRNA levels of PGC-1α and FNDC5 in skeletal muscle both before and after exercise supports that PGC-1α regulates FNDC5 transcription, although the regulation of irisin synthesis from FNDC5 may be a more important step in humans for regulating irisin concentration in blood (Huh *et al.,* 2012).

Reports on measuring irisin in human by Western blot or ELISA have been suggest to be artifacts of poor antibody specificity (Erickson, 2013) even though an earlier study had detected irisin circulating in human plasma using mass spectrometry (Lee *et al.,* 2014). It has been shown that in sedentary individuals irisin levels significantly increased in individuals undergoing aerobic interval training (Jedrychowski *et al.,* 2015).

In mammals, irisin has been shown to have thermogenic actions via the modulation of uncoupling proteins (UCPs) and to affect feeding and energy homeostasis via actions in brain, adipose tissue, liver, muscle and gastrointestinal tract (Butt *et al.,* 2016). FNDC5 is involved in promoting metabolism, immune regulation and affects chronic inflammation in many systemic diseases, including gastric cancer (Shao *et al.,* 2017).

Senile cataract is an age-related, vision-impairing disease characterized by gradual progressive thickening of the lens of the eye. It is the leading cause of treatable blindness worldly. Researchers worldwide have identified factors that may cause cataracts or are associated with cataract development. Besides advancing age, cataract risk factors include ultraviolet radiation, diabetes, hypertension, obesity, smoking, prolonged use of corticosteroid medications, statin medicines used to reduce cholesterol, previous eye injury or inflammation etc. (Raman *et al.*, 2010).

It has been studied that protein destabilization leads to partially unfolded, aggregation-prone intermediates and the formation of insoluble, light-scattering protein aggregates. These aggregates either include or overwhelm the protein chaperone content of the lens. Dysregulation of calcium ion content within the lens is also implicated in cataract formation via activation of calpain proteases. Though they play a role in normal lens development and function their inappropriate over-activation could lead to cataract formation (Wang *et al.*, 1994).

In addition it has been suggested that oxidation may be an initiating contributor in the sequence of events leading to cataract (Cekić *et al.*, 2015). It has been suggested that irisin is a metabolic protective hormone being an oxidative stress marker (Bjørklund, *et al.*, 2017). Therefore, the current study was carried out to analyze irisin levels in serum and aqueous humor of senile cataract patients, in order to analyze that which factors are involved in this pathology.

MATERIAL AND METHODS

It was a cross-sectional, case-control study. The study conformed to the tenets of Declaration of Helsinki (Rennie, 1997). The cataract patients and control subjects were selected from the Christian Hospital, Taxila. Study was formally approved by the Bio-Ethical Committee, Department of Bioinformatics and Biotechnology, International Islamic University, Islamabad, Pakistan (Protocol # BEC-FBAS-BIBT-05) and Christian Hospital Taxila, Pakistan. Written informed consent was obtained from each subject after narrating the study procedures to each one of them.

Cataract patients of both genders were selected through the inclusion criteria; age limit between 40-70 years, no other ocular disease. Subjects aged below 50 and above 70 years with any other ocular complication were excluded from the study. Control subjects of the same age range having no symptoms of cataract were selected for the study. Body weight, duration of disease, history of diabetes and hypertension was recorded in a predesigned study Performa.

Blood (3 mL) was aspirated from cubital vein of all the selected subjects using fresh syringes after cleaning the area with alcohol swab. It was shifted to serum separation tubes (BD Vacutainers, UK) and centrifuged at 10,000 rpm (Eppendorff 5810R, Germany) for 5 min to separate serum. Serum was shifted to micro centrifuge tubes (Axygen Inc, USA).

Aqueous humor samples of the cataract patients were taken from limbus of eye after thorough screening of patient regarding ocular history during cataract surgery with the help of air-filled syringes and transferred to micro centrifuge tubes (Axygen Inc, USA). Both serum and aqueous humor samples were stored at -80 ºC until analysis (Fleury *et al*., 2001).

The *in vitro* quantitative analyses of human irisin concentration in serum and aqueous humor was carried out through Sandwich-Enzyme Linked Immunosorbent Assay (ELISA) using commercially available kit (Elabscience, USA). The micro-ELISA plate provided in kit was pre-coated with an antibody specific to FNDC5. The recommended standard concentrations were as follows: 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781, 0 ng/ml. Standards and samples were added to the appropriate micro-ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for FNDC5 and Avidin-Horseradish Peroxidase (HRP) conjugate was added to each micro plate well successively and incubated. Free components were washed and the substrate solution was added to each well. Only those wells that contained FNDC5, biotinylated detection antibody and Avidin-HRP conjugate appeared blue in color. The enzyme-substrate reaction was terminated by the addition of a sulphuric acid solution and the color turned to yellow. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm using ELISA plate reader (Shenzhen Huisong Technology, China). The OD value was proportional to the concentration of FNDC5 and concentration of irisin was calculated the in the samples by comparing the OD of the samples (Zabibah *et al*., 2019).

All statistical analyses were done through SPSS (version 20.0, Armonk, NY: IBM Corporation). Independent sample T test was applied to compare irisin levels in aqueous humor and serum of cataract patients. Similarly, comparison between serum irisin levels of control and cataract patients was carried out through independent sample t test. Significance level was set at $P < 0.05$

RESULTS

A total of N=50 cataract patients were selected for the study and same number of subjects (N=50) was taken as control. In cataract patient group 26 subjects were males and 24 were females. In control group 23 were males and 27 were females. Average age of patients was 61.08 ± 2.09 years (range 50-70 years). The average age of the control subjects was 61.16 ± 1.09 years (range 50-70 years). The frequency of patients that have 0-3 hours of sun exposure was 20 (40%), 18 (36%) patients had 4-6 hours sun exposure, 7 (14%) patients had 7-9 hours of a sunlight exposure day while 5 (10%) patients had sun exposure of almost 12 hours.

In control subjects, body weight of 27 (54%) subject ranged 40-70 Kg, 19 (38%) subjects had body weight in range 71-100 Kg and 4 (8%) subjects had body weight in range of 101-130 Kg. The body weight of 16 (32%) patients ranged 40-70 Kg, 31 (62%) patients had body weight in range 71-100 Kg and 3 (6%) patients had body weight in range of 101-130 Kg as shown in Table 1.

Only 10 (20%) control subjects were smokers while 40 (80%) were non-smokers. Hypertension was reported in 6 (12%) persons while 6 (12%) were diabetics. In cataract patients, 8 (16%) were smokers while 42 (84%) were non-smokers. Nine (18%) patients were hypertensive and 8 (16%) were diabetic patients.

Duration of cataract was less than 1 year in 10 patients (20%) and 1-3 years in 26 (52%), 4-6 years in 8 (16%) and 7-9 years in 6 (12%) patients. None of the patients was having intraocular pressure more than 20 mm Hg therefore not affected by glaucoma.

The serum irisin levels were significantly high (13.68 ± 2.06 ng/mL) in serum of cataract patients compared to their aqueous humor (1.95 ± 0.39 ng/mL) ($p < 0.0001$; $F=63.797$). The irisin levels were non-significantly different between serum of control (14.83± 6.48 ng/mL) and serum of cataract patients (13.68 ± 1.24 ng/mL) ($p< 0.129$; $F=2.397$) as shown in Table 2.

DISCUSSION

Cataract is one of the leading causes of irreversible blindness worldwide. However, being a complex pathophysiology, senile cataract is yet to be fully understood. It is noticed that oxidation is an initiating step in overall process in the sequence of events leading to cataract. The pathology involves the surge of toxic biochemical reactions leading to aggregation of intracellular protein in eye (Cekić *et al.,* 2010).

The current study was carried out to examine the role of irisin in the pathology of cataract. The irisin levels in the aqueous humor of the cataract patients was significantly reduced compared to the serum of the patients. Irisin, a myokine is produced and secreted by acutely exercising skeletal muscles, is thought to bind white adipose tissue (WAT) cells via undetermined receptors (Bostrom *et al.,* 2012). Irisin has been reported to increase cellular mitochondrial density and expression of uncoupling protein-1, leading to increased energy expenditure via thermogenesis. Irisin may therefore offer potential as a therapeutic approach against metabolic diseases and the associated changes in ageing that are associated with them (Castillo-Quan, 2012). The metabolic role of irisin is characterized by increased energy expenditure and glucose homeostasis. Therefore, it would be logical that irisin and FNDC5 increase in response to aerobic or endurance exercise that are generally characterized by increased oxidative capacity and mitochondrial functions (Boström *et al.,* 2012).

Irisin has been studied to improve homeostasis and lipid profile along with metabolic parameters (Perakakis *et al.,* 2017). In addition, irisin has been studied to reduce the oxidative stress by reducing superoxide and peroixinitrite (Zhu *et al.,* 2015). Currently, irisin levels were significantly lowered in aqueous humor of senile cataract patients however these levels were non-significantly different in the serum of control and senile cataract patients. To our knowledge, this is the first study to examine the association of senile cataract and irisin protein levels in aqueous humor.

The study concluded that the irisin levels in aqueous humor of cataract patients is reduced compared to serum levels however there was no significant difference in the serum levels of control and cataract subjects. Therefore, it is evident that irisin play a role in reducing oxidative stress and thus might be a repressing factor for senile cataract.

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Table 1. Anthropomorphic analyses of control and cataract patients. (Age and body weight are expressed as mean ± standard error of mean).

Parameters	**Control Group (N=50)**	**Cataract Patients (N=50)**
Males	23	26
Females	27	24
M/F ratio	0.85/1	1.08/1
Age (years)	61.16 ± 1.09	61.08 ± 2.09
Body Weight (Kg)	73.52 ± 2.39	77.77 ± 1.94

Table 2. Comparison of irisin levels (ng/mL) in control serum and serum and aqueous humor of cataract patients.

Samples	**Serum control (Mean ± SEM)**	**Serum patients (Mean ± SEM)**	**Aqueous humor patients (Mean ± SEM)**
Irisin levels (ng/mL)	14.83 ± 6.48	13.68 ± 2.06	1.95± 0.39*

*Shows $p < 0.0001$ significantly lowered than serum levels in patients; SEM: Standard error of mean

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