Original Article

Association of Serum 8-isoprostaglandin $F_{2\alpha}$ Levels with Glycemic Control in Type 2 Diabetes Patients with Senile Cataract

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Background : Lipid Peroxidation (LPO) plays a major initiative factor of cataractogenesis in both age-related or senile cataract and Diabetic cataract. Recently, 8-isoprostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) is a reliable biomarker of in-vivo LPO and used as potential indicator of oxidative stress. However, serum 8-iso-$PGF_{2\alpha}$ concentration and its association with glycemic control (HbA1c) in the pathogenesis of diabetic cataract subjects are still unknown.

Objectives : The present study was designed to estimate 8-iso-$PGF_{2\alpha}$ and antioxidant enzymes levels in serum of Type 2 Diabetes Mellitus patients with senile cataract compared to healthy individuals without cataract as control. To assess the magnitude of the association between 8-iso-$PGF_{2\alpha}$ and glycemic status in diabetic cataract.

Materials and Methods : 60 Diabetic Senile Cataracts (DSC) and 60 healthy individuals without cataract in the age group between 45-75 years of both genders. 8-iso-$PGF_{2\alpha}$, Superoxide Dismutase [Cu-Zn] (SOD3) and Catalase (CAT) concentration were estimated in serum by ELISA method.

Results : The mean concentration of 8-iso-$PGF_{2\alpha}$ was significantly increased (541.6±142.7 pg/ml, $p<0.001$) and mean concentration of SOD3 (102.1±32.8 ng/ml, $p=0.007$) and Catalase (1005±274.5 IU/ml, $p<0.001$) were significantly decreased in serum of diabetic senile cataract when compared to healthy individuals without cataract (control). A negative correlation between serum 8-iso-$PGF_{2\alpha}$ and SOD3 and positive correlation between serum 8-iso-$PGF_{2\alpha}$ and fasting blood glucose were observed in Diabetic Senile Cataracts.

Conclusion : The present findings indicate that increased 8-iso-$PGF_{2\alpha}$ is associated with oxidative stress which plays a significant role in the pathogenesis of cataract in diabetic patients.

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***Key words* : 8-isoprostaglandin $F_{2\alpha}$, Senile cataract, Diabetic cataract, Superoxide dismutase, Catalase.**

Cataract is defined as cloudiness or opacity of the eye lens and the leading cause of blindness Worldwide. Oxidative stress play an important role in the pathogenesis of cataract formation in both senile cataract (Age-related cataract) and hyperglycemia induced cataract (diabetic cataract)[1]. Oxidative stress represents an imbalance between pro-oxidant and antioxidant status which leads to generation of free radicals resulting in cellular damage[2,3]. Lipid Peroxidation (LPO) levels are used as a vital marker of oxidative stress[4]. Increasing evidence indicates that free radical induced lipid peroxidation represent one of the primary pathogenic factors of ocular changes in senile cataract[4-7]and diabetic cataract patients[8]. Though several reports are available on oxidative stress markers in cataracts subjects, the results are conflicting as there is paucity in specificity and sensitivity[9]. Recent studies have reported that 8-isoprostane F2-alpha (8-iso-$PGF_{2\alpha}$) is the most stable product and reliable biomarker of in vivo lipid peroxidation and oxidative stress[10,11]. However, there are very few studies with reference to 8-iso-$PGF_{2\alpha}$ concentration in diabetic cataract patients. The relationship between 8-iso-$PGF_{2\alpha}$ and hyperglycemia is still unknown. Hence the present study was designed to investigate the lipid peroxidation, 8-iso-$PGF_{2\alpha}$ and antioxidant enzymes levels in serum of Type 2 Diabetes Mellitus patients (T2DM) with senile

Editor's Comment :

- The association between oxidative stress and glycemic index is now a matter of concern in patients with diabetic cataract and this would serve the patient community to heal better if they are tested for markers of oxidative stress whenever they check for glycemic index for overall wellness in every individual who have cataract.

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cataract compared to healthy individuals without cataracts.

Materials and Methods

Study design : This observational comparative study included 60 Healthy individuals without cataract subjects (Group I) and 60 T2DM patients with senile cataract (Group II) aged between 45 to75 years of both genders. The study was conducted in the Department of Biochemistry in collaboration with Department of Ophthalmology in a Tertiary Care Hospital, Puducherry from June 2018 to July 2019. The subjects were selected based on inclusion and exclusion criteria from Ophthalmic OPD. The study was approved by Institutional Human Ethics Committee (IHEC Project No: Faculty Project/2017/05/16) and informed consent was obtained from all the study subjects. Necessary clinical parameters were assessed by a physician and then cataract was defined on the basis of slit lamp examination by an ophthalmologist. Lens opacities classification system (LOC III) was used for grading the cataract. The purely nuclear type of cataractous lens was obtained from the patients during Small Incision Cataract Surgery (SICS) followed by Intraocular Lens (IOL) implantation.

Inclusion criteria :

Group I : Healthy individuals without cataract subjects recruited from the Ophthalmology OPD for eye check-up.

Group II : T2DM patients having more than 5 years of duration who are under treatment of oral hypoglycemic drugs with senile cataract were included in this study.

Exclusion criteria : Patients having history of Steroid intake, Ophthalmic disease, Renal disease, Autoimmune disorders, Hypothyroidism, Hyperthyroidism, Hepatic disease, Traumatic or Toxic cataract, Alcohol, Smoking and other systemic disorders were excluded from study.

Sample collections : Blood samples were drawn and placed in EDTA and sodium fluoride-Potassium oxalate vials after 12 hours of fasting. The plasma sample was separated by centrifuging at 3500 rpm for 15 minutes. Plasma sample was used for the estimation blood glucose. Whole blood was used for the estimation of Glycated Haemoglobin (HbA1c).

Biochemical analysis in blood : Fasting plasma glucose was estimated by glucose oxidase – Peroxidase (GOD–POD) method using clinical chemistry Beckman Coulter Olympus AU400 auto-analyzer. Glycated haemoglobin (HbA1c) was estimated in whole blood by HPLC method using Biorad D10 HbA1c analyser.

Estimation of 8-isoprostaglandin $F_{2\alpha}$, SOD3 and Catalase in serum:

8-isoprostaglandin F2 (8-iso-$PGF_{2\alpha}$), Superoxide dismutase [Cu-Zn] (SOD3) and CAT were estimated by sandwich ELISA (Bioassay technology Lab, Shanghhai, China). Samples and standards were added to the microtitre plates pre-coated with anti-8-iso-$PGF_{2á}$, anti-SOD and anti-CAT antibody. After the removal of unbound proteins, anti-8-iso-$PGF_{2\alpha}$, anti-SOD and anti-CAT antibodies conjugated with streptavidin HRP were added to the micro titre plate. After washing, enzyme bound was assayed by the addition of a chromogenic substrate, TMB. The reaction was terminated by adding stop solution. The quantity of 8-iso-$PGF_{2\alpha}$, SOD and CAT in the lens samples were calculated from the standard curve by measuring absorbance at 450 nm.

Statistical analysis :

The results were expressed as Mean ± Standard Deviation (SD). Data was analysed using JASP 8.4. The statistical significant differences between groups were analysed using the Student's t-test. Spearman's correlation coefficient (rho) was carried out for the assessment of association between the variables. Multiple linear regression analysis was performed to assess independent relationship between 8-iso-$PGF_{2\alpha}$, antioxidant enzymes and glycemic status. A p value of < 0.05 was considered as statistically significant.

Results

The general characteristics of the healthy individuals without cataract as control group (group I) and Type 2 Diabetics with senile cataract patients (group II) were shown in Table 1.We found that cataract formation occurs at an early age group in patients with diabetic cataract (59.18±7.51), p=0.016 when compared to healthy individuals without cataract (62.57±7.56).

Fasting Blood Glucose was significantly elevated in group II (157± 62), p<0.001 when compared to group I (87±14.00). Similarly HbA1c was also significantly elevated in group II (7.7±1.88), p<0.001 in comparison

Table 1 — *General characteristics of Group I and Group II*

Parameters	Group I (Healthy individuals without cataract) (n=60)	Group II (Type 2 DM patients with senile cataracts) (n=60)	p Value
Age (Years)	62.57 ± 7.56	59.18 ± 7.51	0.016*
Gender (Female/Male)	36/24	32/28	-
Duration of diabetes(years)	-	8.27 ± 0.37	-

* p<0.05 significant, ** p<0.01, ***p<0.001

with group I (5.1 ± 0.44) as shown in Table 2.

Table 3 shows the mean levels of serum 8-iso-$PGF_{2\alpha}$, SOD3 and catalase in group I and group II. The mean concentration of serum 8-iso-$PGF_{2\alpha}$ was significantly elevated in group II (541.6±142.7), p<0.001 when compared to group I (413.4±168.6).We found that serum SOD3 was significantly lower in group II (102.1±32.8) in comparison to group I (127.7±51.3), p=0.007. Similarly catalase activity in serum of group II (1005±274.5, p<0.001) subjects was significantly reduced when compared to group I (1575 ± 655.3).

In addition, there was significantly positive correlation between 8-iso-$PGF_{2\alpha}$ and fasting blood glucose (r= 0.299*; p=0.02) and negative correlation between 8-iso-$PGF_{2\alpha}$ and SOD (r= -0.263*; p=0.04) in serum of Type 2 diabetic with senile cataract patients (Fig 1 & 2).

Table 4 shows the results of multiple linear regression analysis for group II (diabetic cataract) with 8-iso-$PGF_{2\alpha}$ as dependent variable. Fasting blood glucose and SOD3 showed positive and negative influence with respect to oxidative stress marker.

Discussion

Cataract is one of the major causes of visual impairment and blindness in diabetic population[12].

Table 2 — *Fasting blood glucose and HbA1c levels between Group I and Group II*

Parameters	Group I (Healthy individuals without cataract) (n=60)	Group II (Type 2 DM patients with senile cataracts) (n=60)	p Value
Fasting plasma glucose (mg/dL)	87 ± 14.00	157 ± 62	<0.001***
HbA1c (%)	5.1 ± 0.44	7.7 ± 1.88	<0.001***

* P<0.05 significant, ** p<0.01, ***p<0.001

Table 3 — *Serum 8-iso-$PGF_{2\alpha}$, SOD3 and Catalase levels between Group I and Group II*

Parameters	Group I (Healthy individuals without cataract) (n=60)	Group II (Type 2 DM patients with senile cataracts) (n=60)	p Value
8-iso-$PGF_{2\alpha}$(pg/ml)	413.4 ± 168.6	541.6 ± 142.7	<0.001***
SOD3 (ng/ml)	127.7 ± 51.3	102.1 ± 32.8	0.007**
Catalase(IU/ml)	1575 ± 655.3	1005 ± 274.5	<0.001***

* P<0.05 significant, ** p<0.01, ***p<0.001

Table 4 — *Multiple linear regression analysis for type 2 DM with cataract patients (Dependent variable: 8-iso-$PGF_{2\alpha}$)*

Model	Coefficients			t	p Value	95% CI	
	Unstandardized coefficients β	Standard error	Standardized coefficients β			Lower	Upper
FBS	0.800	0.275	0.349	2.912	0.005	0.250	1.350
SOD3	-1.011	0.521	-0.232	-1.942	0.057	-2.054	0.031

* P<0.05 significant, ** p<0.01, ***p<0.001

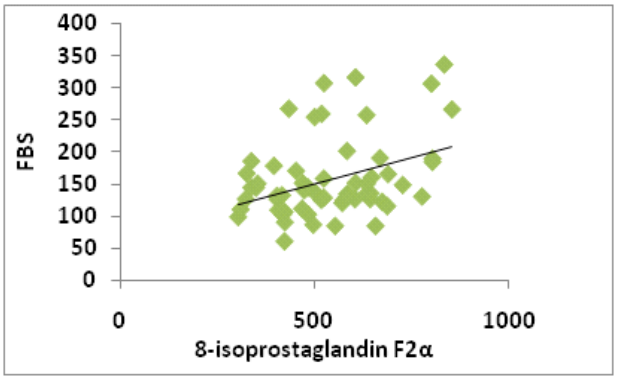

Fig 1 — Association between 8-iso-$PGF_{2\alpha}$ and Fasting Blood Glucose (FBS)

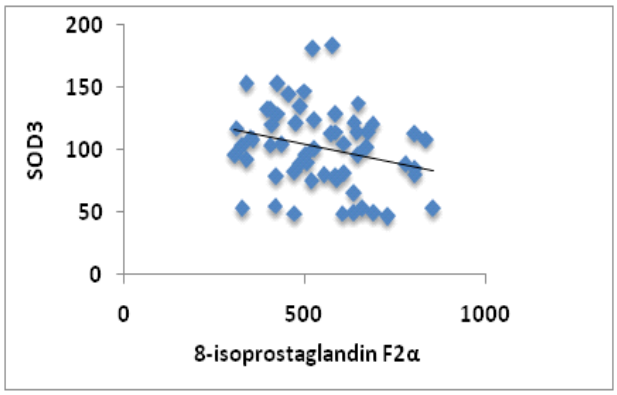

Fig 2 — Association between 8-iso-$PGF_{2\alpha}$ and SOD3

Several lines of evidence suggest that cataract formation occurs more often at an early age and advances much faster in diabetics when compared with non-diabetic subjects[13,14]. Multiple pathogenic mechanisms have been proposed to explain the cataract formation in Diabetes Mellitus such as increased sorbitol concentration, abnormal glycosylation of proteins and enhanced free radical production in lens, but still not fully understood[15-17]. Among several risk factors, oxidative stress plays a major role in the development of cataract in diabetic subjects. However, the exact mechanism by which oxidative stress contributes to development of cataract remains unclear.

Lipid Peroxidation (LPO) is caused by an imbalance between the free radical production and the antioxidant defenses and plays a significant role in the cataractogenesis[18]. Lipid peroxidation is the major marker of oxidative stress and the oxidative damage can be measured by estimating the primary or secondary LPO end-products. The Primary LPO end-products are conjugated dienes and lipid hydroperoxides, while

secondary end-products which include Thiobarbituric Reactive Substances (TBARS), gaseous alkanes and F2- isoprostanes (F2-IsoPs). Among the LPO markers, measurement of 8-iso-$PGF_{2\alpha}$ has advantage over the other markers. Since they are biologically active, stable, specific and easily identified in biological fluids and tissue and has been widely used as a valid marker of oxidative stress[19–21].

We found that 8-iso-$PGF_{2\alpha}$ levels in serum of Diabetic cataract patients were significantly elevated when compared to healthy individuals without cataract. In line with our findings, Amena Rahim et al have shown that mean concentration of 8-isoprostaglandin $F_{2\alpha}$ level in aqueous humor was significantly higher in diabetic patients with cataract than age matched senile cataract patients[22]. Another study also reported that the mean concentration of 8-iso-$PGF_{2\alpha}$ in aqueous humor was approximately 5 times higher in patients with exfoliation syndrome and cataracts[23]. Also few studies have shown the elevated lipid peroxides levels in diabetic cataractous patients when compared with senile cataract[8,24]. Although very limited studies are available with reference to 8-iso-$PGF_{2\alpha}$ levels in cataract subjects, our findings were supported with these reports. It was also shown that hyperglycemia generate excess free radicals due to auto-oxidation of glucose and glycosylation of proteins. Thus hyperglycemia induced free radical attacks the membrane lipids especially polyunsaturated fatty acid resulting in increased lipid peroxidation product, 8-iso-$PGF_{2á}$ which plays a major role in the development of microvascular complications in diabetic subjects. Hence the accumulation of 8-iso-$PGF_{2\alpha}$ in cataract lens is a key factor in the early development of cataractogenesis in diabetic subjects when compared to healthy controls.

In the present study, fasting blood glucose level and HbA1c were significantly elevated in diabetic cataract subjects. Also there was significant positive correlation between 8-iso-$PGF_{2\alpha}$ and fasting blood glucose levels in diabetic cataract subjects. Our results suggest that hyperglycemia in diabetic subjects may contribute to oxidative damage in lens via increased 8-iso-$PGF_{2\alpha}$ levels which could be a causative factor in early development of cataract formation in Type 2 Diabetic patients.

Human lens has several defense mechanisms against the oxidative damage caused by ROS. The major protective enzymes in the lens are Superoxide Dismutase (SOD), Catalase (CAT)[25] and Glutathione Peroxidase (GSH-Px)[26]. SOD is a chain breaking most predominant antioxidant enzyme in lens, which acts by removing the toxic superoxide radical, $O_2^{"}$ by converting it into H_2O_2 which in turn, can be decomposed by CAT and or GSH-Px[27]. In vitro and in vivo studies have shown that SOD has protective properties against cataract development in Diabetes Mellitus[8,28-30]. But some studies have reported the contradictory findings on SOD levels and other anti-oxidant enzyme levels. We found reduced anti-oxidant enzyme activities in diabetic patients with senile cataract as illustrated by decreased SOD3 and CAT activity. These findings were consistent with previous report in which lens copper, zinc, SOD and catalase levels were significantly lower in the diabetic patients when compared to senile cataract subjects[8]. Similar studies have shown that serum and erythrocyte SOD levels were decreased in diabetic subjects with cataract when compared with senile cataract subjects[6,30]. We also observed negative correlation between 8-iso-$PGF_{2\alpha}$ and SOD in serum of Type 2 diabetic with senile cataract patients. Thus it is evident that Hyperglycemia and Lipid peroxidation as shown by elevated levels of 8-iso-$PGF_{2\alpha}$ may contributed to the reduced anti-oxidant enzyme levels of SOD3 and CAT in the current study. The reduced anti-oxidant enzyme activity of SOD3 and CAT in serum leads to elevation of H_2O_2 and superoxide radicals which may contribute to oxidative damage of lens and associated with early development of cataract formation in Type 2 Diabetic patients.

Conclusion

The cardinal concept in the present study indicates that oxidative stress was significantly higher in serum of diabetic cataracts which is evidenced by elevated levels of 8-iso prostaglandin F2α and decreased antioxidant enzymes SOD3 and CAT. In diabetic subjects, hyperglycaemia induces increased production of ROS which leads to lipid peroxidation resulting in oxidative stress and depletion of antioxidant enzymes in the lens tissue. These could have played a significant role in the pathogenesis of diabetic induced cataract.

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