Cataract is a light-scattering disorder of the crystalline lens occurring due to opacification. Despite surgical treatment, it remains as an important cause of visual impairment worldwide. As per World Health Report of 2010, 50 million people are estimated to have turned blind due to age-related cataract (ARC). Prevalence of cataract is observed to be high in India as compared to other populations. Survey by Andhra Pradesh Eye Disease Study claimed cataract among aged as a leading cause for blindness accounting to 44% of the population. Based on the anatomical location of opacity, morphology of opacification, and its onset and progression cataracts are diagnosed into 3 different types namely nuclear, cortical and posterior subcapsular. Nuclear cataract is the most frequent form of cataract that occurs among elderly and the opacity yellow in colour develops in the nuclear region of the lens. Cortical cataract develops in the cortical region of lens and it becomes significant when the opacities extend from periphery to centre. Posterior subcapsular cataract tends to occur in younger patients than cortical or nuclear cataracts and the opacities develop at the back of the lens capsule. Diagnosis of the types of cataracts is done by Lens opacities classification system (LOC-III) – a highly reproducible classification system followed by the ophthalmologists worldwide.

Studies in the past decades have provided evidence for multi-factorial basis for the condition with interaction between the genes and environmental factors, dietary habits and exposure to UV light etc. Recently the Cat-Map, a reference database developed for cataracts reported involvement of genes like CRYAA, GJA8, HSF4, EPHA2, GALT, SLC16A12, GALK1 and FTL in the development of ocular opacity. IDO gene has been added to this list recently.

Among several aetiological factors, exposure to UV light is identified as a major risk conferring factor for ARCs for several years. Nature has a mechanism of protecting the lens from losing its transparency by the ability of cornea to cut off UV-B light with absorption below 295nm. But the UV-A light that is absorbed at 315-400nm can reach the lens tissue and affect its transparency and normal vision. Low molecular weight compounds referred as UV filters [like kynurenine (KN) and 3hydroxy kynurenine (3-OHKN) formed during tryptophan catabolism] are synthesized in the lenticular epithelial
cells which absorb major portion of the UV light (~90-95%, at 360-370) and protect the lens from UV induced photo damage.

Biochemically lens is composed of several components including proteins, enzymes and elements. Crystallines (α, β and γ) form the major portion of the lens and share a greater role in maintaining its transparency. Post translational modifications of crystalline in the lens involving oxidation, cross-linking, truncation and aggregation are known to result in the accumulation of modified proteins in the lens with age, adding colour, fluorescence and insolubility. Other cause of protein modifications includes the thermal or photochemical reaction of UV filters formed during tryptophan catabolism. The UV filters get covalently attached to the lens proteins, influence protein functionality and increase their susceptibility to UV light leading to lens opacification. With ageing, the levels of free UV filters in the lens are found to be decreased at a rate of 12% per decade accompanied by increase in the levels of UV filter modified proteins leading to lens opacification.

IDO (Indoleamine 2,3-dioxygenase) is the first rate limiting enzyme involved in tryptophan catabolism leading to UV filter synthesis. It also acts as a free radical scavenger by utilizing superoxide anion (\( \text{O}^{2-} \)) generated in lens for oxidative cleavage of the pyrrole ring of tryptophan to N-formyl-L-kynurenine. The IDO protein is encoded by the gene designated as IDO or INDO (OMIM No.147435) and is located on chromosome 8p12-11. IDO is a single copy gene comprising 10 exons spanning 15 kb and codes for a protein of 403 amino acids. It also has promoter of 1.3 kb located at 5’ region of exon1.

IDO needs several inflammatory mediators for its production including interferons such as IFN-γ. The two polymorphisms, 12 CA repeat and +874 A>T at IFN-γ, have been associated with risk for certain conditions such as acute kidney rejection, aplastic anemia, tuberculosis, symptomatic parovirus infection and recurrent pregnancy loss. IFN-γ+874A>T polymorphism lies within a binding site for the transcription factor NF-kB, which shows an allele-specific binding pattern. NF-kB site induces IFN-γ expression and the presence of T and A alleles of +874 A>T polymorphism are related to high and
low $IFN-\gamma$ expression respectively, which in turn influences the $IDO$ activity proportionally.

Based on these observations a study on sequence variations in the $IDO$ gene and $IFN-\gamma$ polymorphism in cataract patients and evaluation of risk causing epidemiological factors is expected to throw light on the mechanisms related to cataract formation. It also opens new avenues to improved therapy to delay or prevent the occurrence of the condition.

**OBJECTIVES**

Keeping in view the high prevalence of ARC, lack of sufficient information on the contribution of genetic factors causing susceptibility to ARC in Indians, the present investigations were undertaken with the following objectives:

- To assess the contribution of epidemiological factors in the expression of ARCs such as - sex, age, age-at-onset, body mass index, obesity, dietary habits, smoking, alcohol consumption, familial history.
- To screen different types of age-related cataracts (Nuclear-NC, Cortical-CC and Posterior subcapsular- PSC) for variations in all the 10 exons with flanking intronic boundaries and promoter of $IDO$ gene in comparison to healthy normal controls by SSCP analysis.
- To characterize the sequence variations (SNPs) detected by SSCP through sequence analysis, RFLP genotyping and Bioinformatic tools.
- To study the expression of the novel sequence variations identified in exons of $IDO$ gene.
- To evaluate the risk of $+874A>T$ polymorphism of the $IFN-\gamma$ gene that induces $IDO$ gene in developing different types of age-related cataracts

Screening for sequence variations in $IDO$ gene along with functional analysis and risk estimations for $+874 A>T$ polymorphism of the $IFN-\gamma$ gene that induces IDO production is expected to provide better understanding of the aetiopathogenesis of ARC at molecular
level. The study planned is also expected to identify the epidemiological risk factors that may contribute to the susceptibility to ARCs.

MATERIALS AND METHODS
The present attempt is a case-control study of different types of ARC that was conducted between “2008-2012”. About 680 ARC cases were ascertained for various epidemiological parameters and also to study the association of the polymorphism +874 A>T of *IFN-γ* gene. Of these cases 331 diagnosed with different types of ARCs (NC-110, CC-110 & PSC-111) and 210 healthy normal controls were screened for the variations in the *IDO* gene and its promoter.

The patient and control subjects participated in the study were explained about the purpose and outcome of the study and only those who gave their consent to provide the blood samples and demographic history were considered. The study was approved by the institutional ethical committee following Helsinki guidelines.

**Subjects and source:**

**Cases:** In all, 331 age-related cataract patients were studied with the diagnosis of Nuclear cataract (NC) in 110, Cortical cataract (CC) in 110 and Posterior subcapsular cataract (PSC) in 111 cases. Patients studied were recruited from among the inpatients registered for surgery of cataracts at Sarojini Devi Eye Hospital and Institute of Ophthalmology, Hyderabad, India. Diagnosis for the type of cataract was done based on the lines of Lens Opacities Classification – III (LOC- III) using slit lamp.

**Controls:** 210 normal healthy individuals without cataract were selected at random as controls for the study by personal contacts, house visits and from among the employees of government and private organizations with the provision for annual health check up.
**Abstract**

**Inclusion and exclusion criteria:**
Only patients with primary cataracts were included in the present study and those arising due to trauma, action of toxins, inflammations and degenerative ocular diseases were excluded. In addition, patients with associated conditions like diabetes, hypertension, myopia, glaucoma, thyroid syndrome and those using cataract inducing medications (like steroids) were not considered. Controls subjects were also without the history of cataract, other ocular diseases and conditions like diabetes, hypertension, thyroid disorders etc. at the time of investigation.

**Collection of data and blood samples:**
From all the patients and controls, information pertaining to sex, age, age at onset, duration of disease, type of cataract, information on habits, diet and detailed medical history along with three generation pedigrees were collected using a specified proforma. Venous blood samples (5ml) were collected from all the patients and controls in EDTA vaccutainers for isolation of DNA by rapid non enzymatic method (Lahiri and Nurnberger, 1991). The DNA samples isolated from subjects were used to study the sequence variations in *IDO* gene.

The data generated was evaluated for:

**(I) Association of Epidemiological factors with ARC**
In the present study about 680 subjects with ARC were studied for association with epidemiological parameters like

- Sex
- Age
- Age at onset
- Body Mass Index (BMI)
- Familial History
- Diet (vegetarian and non-vegetarian)
- Habits (smoking, alcohol consumption)
II) Screening for sequence variations

The change in nucleotide sequence of a gene may be caused due to a mutation or change that arises due to polymorphism. The present study was carried out to screen for SNP variations in all the 10 exons and promoter of *IDO* gene by amplification of the *IDO* gene sequences by Polymerase Chain Reaction (PCR) using 17 sets of primers. PCR amplified samples were screened for sequence variations (SNPs or mutations) by the techniques specified below.

**a) Single-Strand Conformation Polymorphism (SSCP):** SSCP analysis was carried out for the detection of variations in

- All 10 exons including exon-intron boundaries of *IDO* gene
- 1.3 Kb promoter region of *IDO* gene

Although SSCP is one of the most economic and widely used technique in gene screening studies, its sensitivity of detecting a sequence change ranges from 70-80% and hence there could be a possibility of missing few variations as compared to direct sequencing. As the sequencing of 541 subjects is highly expensive, SSCP technique was employed in the present study.

**b) Sequencing:** the variant samples detected by SSCP were sequenced by ABI 3100 automated DNA sequence analyzer to know the type of nucleotide change/substitutions.

**c) Restriction Fragment Length Polymorphism (RFLP):** RFLP was designed for the variants identified to facilitate direct genotyping of all samples without the need for sequencing which works out expensive if large samples have to be screened.

The variants identified and the restriction enzymes used to design the RFLP analysis were

- c.422+90 G>A in intron 4 using Hha I
- c.596 _597 delins TT in exon 7 using Hha I
- c.822 C>T in exon 9 using Aat II
- c.-979 G>A in the promoter region using BseM I
- c.-471T>G using AciI
III) Amplified Refractory Mutation System (ARMS): ARMS PCR technique was used for genotyping $IFN-\gamma +874$ A>T polymorphism

IV) Expression Studies
Screening for variations in $IDO$ gene revealed a novel mutation (p.A199V) in exon-7 of $IDO$ gene, which was further analyzed for its functional variations in comparison to wild-type IDO protein.

The steps involved in the analysis were:
- Generation of IDO cDNA
- Generation of wild type IDO clone
- Generation of mutant clone (P199V) by site directed mutagenesis
- Expression and purification of recombinant IDO fusion protein
- Assay of IDO activity to compare wild type and mutant proteins

For generation of IDO cDNA, mRNA was isolated from human lens epithelial cells and IDO cDNA was amplified using gene specific primers. The amplified cDNA was ligated into the pGEX-KG vector. Then the recombinant wild type vector pGEX-KG (WT) was checked for successful incorporation of IDO cDNA through sequencing. Mutant clone was generated by using site directed mutagenesis kit (Geneart® Site-Directed Mutagenesis kit, U.S) and recombinant wild type vector as template. The mutant clone pGEX-KG (MT) generated was sequenced to check for the incorporation of mutant sequence. The clones thus generated were transformed into Top10 cells and later into BL21 cells and induced by IPTG for over expression of IDO wild type and mutant proteins. The wild and mutant proteins were isolated by agarose affinity column chromatography. Assay of IDO activity was carried out by incubating 125ng of enzyme with different concentrations of the substrate (tryptophan) for 1 hr followed by measurement of OD at 480nm.

For the variants identified in promoter region quantitative studies or familial segregation studies could not be done because the families were not available for drawing fresh blood samples. As all the cases in the present study were inpatients of government hospital and were from different districts so we couldn’t trace all the members of the families.
(V) Statistical Analysis

i. The data was analyzed for epidemiological parameters using descriptive statistics and tests of significance (chi square test) to compare the stratified groups of 680 ARC patients and 210 controls. The contribution of independent variables for predicting the onset of ARC was studied by Logistic regression analysis.

ii. The $IFN-\gamma +874\; A>T$ polymorphism analyzed in patients with different types of ARCs (680) and controls (210), were analyzed by Chi-square test of significance and Odds ratios (OR) with 95% confidence Intervals (CI) were computed to estimate the risk of specific genotypes for developing different types of cataracts.

VI) Bioinformatic Analysis

Different bioinformatic tools were used to interpret the significance of the variations found in $IDO$ gene among the patients. They were: (a) CLUSTAL X - to check the homology of variant in different species; (b) Polymorphism Phenotyping (Polyphen)- to predicts possible impact of an amino acid substitution on the structure and function of a human protein; (c) Sorting Intolerant From Tolerant (SIFT) - to predict the effect of sequence changes on the protein function based on homology search and the physical properties of amino acids; (d) Triton V 4.0.0 package-for protein modeling and (e) Human splicing finder (HSF) - to predict the effect of variation on splicing signals to study the functional characterization of variants identified in the present study.

RESULTS

In the present study attempt was made to evaluate the contribution of epidemiological factors like sex, age, age-at-onset, obesity, habit of smoking, alcohol consumption, dietary habits and family history to the development of ARCs. During the past decade, focus on these factors and also the genes causing susceptibility to cataracts has progressed and provided insights into the mechanisms of cataractogenesis. Nevertheless, aetiopathogenesis of ARC still remains incompletely understood.
I. EPIDEMIOLOGICAL PARAMETERS:

a) The baseline characteristic features and the association of cohorts under each risk causing epidemiological parameters for cataracts revealed the following observations

1. There was a high preponderance of females with ARC (54.3%) in general, showing statistically significant difference when compared to male patients (45.7%; $\chi^2$: 18.82; p: 0.00).

2. The mean±SD for age, age-at-onset and BMI of ARC patients were 58.66±0.40; 57.45±0.40 and 22.5±0.11 respectively, and for controls the mean±SD for age and BMI were 49.75±0.5 and 23.4±0.23. Mean values for age and BMI showed significant difference between cases and controls ($p\leq0.0001$), the mean BMI being lower and mean age higher in patients as compared to controls.

3. In general it was observed that female subjects and subjects above 50 years of age, those with low BMI (<25.0), non-vegetarian food habit and males with the habit of smoking were at greater risk for the development of age-related cataracts. The risk estimates for these parameters can be used as empiric risk figures for our population.

b) An attempt was made to study the association of ARCs with different cohorts for each epidemiological risk factor of the patients.

1. Risk estimates for different cohort groups in two sexes showed high risk for females with early onset (<50 years), with low BMI (<25.0) and non-vegetarian food habits. While males were at risk when they were ≥50 years of age, with low BMI, with the habit of smoking and non-vegetarian food intake.

2. Considering frequency distribution of cohorts in different age groups, about 2/3 of cases in age group 50-69 years showed risk for ARCs in general while the risk was high for female patients in middle age (30-49 years) and male patients in older age groups (>70).
3. Considering age at onset, significantly high frequency of female patients (63.8%) were found as compared to male patients (36.2%) with early onset of cataract suggesting 1.8 times higher risk in females for developing cataracts below 50 years. In contrast among late onset cases the difference was not significant between the frequencies among females (51.8%) and males (48.2%).

4. Regarding Body Mass Index (BMI) there was a significantly high frequency of female cases (55.0%) with low BMI (< 25.0) as compared to male cases (45.0%) and controls (34.2%). This suggests that females may be at greater risk than the males when BMI is low (< 25.0). The patients in general with low BMI (<25.5) and in elder age group with non-vegetarian dietary habit showed greater susceptibility to develop age-related cataracts.

5. Considering familial history, female patients with positive family history (55.1%) were high in frequency as compared to male patients (34.9%) and also the control females (46.3%; $\chi^2$: 4.45; p: 0.03). Among the cohorts in the familial group of patients the risk of developing cataract was more for female sex, subjects above 50 years of age, with low BMI, non-vegetarian food habit and males with the habit of smoking.

6. Among smoker males a high frequency of male patients was observed to be smokers (49.2%) as compared to controls (38.6%). Smokers with positive family history and non vegetarian dietary habits were predicted to be at risk for the development of age-related cataracts.

7. Among the alcoholic subjects who were all males the risk of developing cataract increased along with the habit of smoking and non vegetarian diet. This emphasizes the need to control the habit of smoking and alcohol consumption for the management of age-related cataracts.

8. Logistic regression analysis also provided evidence for the age ≥50 years, female sex, low BMI and non-vegetarian dietary habits as the best independent variables that can be used to predict the onset of the condition.
II) SEQUENCE VARIATIONS DETECTED IN IDO GENE

Screening for variations in IDO gene revealed 7 variants - 4 in exons and intronic boundaries and 3 in promoter region.

a) Sequence variations detected in the exons with flanking intronic region of IDO gene

Observations made from screening for variations in all the 10 exons and intronic boundaries of IDO gene revealed the presence of 4 genetic variants, two in exons (exon 7 & 9) and two in intronic region (intron 4 & 8). The two variants found in exons were novel and other two intronic variants were already reported in SNP database.

Novel variants

1. Screening for variations in exon 7 and intronic boundaries showed heterozygous banding pattern in two of the cases (one with NC and one with CC) studied and none among controls. The two probands with this variation were males with an age at onset of 59 and 45 years respectively. Sequencing of the samples revealed the presence of a novel variation that was interesting i.e. the deletion and insertion of two nucleotides in succession were found at position c.596 and c.597 of IDO gene which are registered in the NCBI SNP database as rs267606590.

The deletion (CG) and insertion (TT) of two successive nucleotides at this position causes codon change leading to substitution of alanine (A) at position 199 by valine (V) in variants. This variation p.A199V showed loss of site for HhaI enzyme.

Alignment of IDO amino acid sequence from several species using CLUSTAL X showed high conservation for amino acid Alanine at 199 position. SIFT and PolyPhen tools predicted “probable damaging effect” with PSIC score of 1.53 by the variant on protein function and with a significant SIFT score of 0.00. Superimposition of IDO mutant and wild type proteins (2DOT) by Triton package showed RMSD value of 1.19, which indicated wide variation between the wild type and mutant protein structure.
2. Sequencing of the samples showing mobility shift on SSCP analysis revealed C to T transition in heterozygous condition at c.822 of \emph{IDO} gene. c.822C>T is a novel synonymous mutation coding for aspartic acid at 274\textsuperscript{th} position of the protein. As per codon usage table the variant showed 46\% of accessibility during protein synthesis. This variation resulted in the loss of restriction site for the enzyme Aat II. HSF predicted the break of potential branch point in variants, and ESE and EIE predicted destruction of enhancer site with a score of -100.

**Known variants**

3. Samples showing variation by SSCP analysis on sequencing showed c.422+90 G>A (rs4613984) transition in intronic region lying between exon 4 and 5 in six samples (3 with NC and 3 with PSC) that correlated with homo and heterozygous patterns. This transition caused loss of HhaI site resulting in homozygosity in one patient (with NC) and heterozygosity in 5 patients (2 with NC and 3 with PSC). This known variation was not found in controls. HSF did not predict any effect on potential splice site/branch point with this variation, but showed destruction of two enhancer sites with a score of -100 and creation of a new silencer motif with a variation score of -16.23.

4. A known variation rs3214412 showing (-/CAA) deletion in intron 8 was found both among cases and controls of the present study indicating the polymorphic nature of the variation found. The frequency of heterozygotes (ID) was high in PSC (10.8\%) as compared to NC (7.3\%), CC (5.5\%) cases and controls (5.7\%). Estimate of odds ratio showed protection for wild type allele ‘I’ (OR= 0.43; 95\%CI =0.18-1.02; P=0.03) in PSC cases while variant allele ‘D’ showed 2 fold risk (OR= 2.28; 95\%CI =1.01-5.38; P=0.03) for developing PSC. The bioinformatic tools, EIEs and PESE octomer from Zhang and Chasin predicted destruction of enhancer site with a variation score of -100.

5. Screening for variations in exons 1, 2, 3, 5, 6 and 10 didn’t show any mobility shift by SSCP analysis suggesting the absence of detectable variations in them.
b) Sequence variations detected in the promoter of IDO gene

Screening for variations in promoter of IDO gene revealed the presence of 3 variants - two novel (c.-979 G>A & c.-471 T>G) and one known variant (c.-738 A>G).

Novel variants

1. The novel variant c.-979 G>A was found in two of the cases (1 with NC & 1 with PSC), resulting in the loss of GATA2 transcription factor binding site. This variation created a restriction site for BseMI enzyme.

2. The presence of another novel variant c.-471 T>G in the promoter was identified in 3PSC cases, which created two SPIB and ETS1 transcription factor binding sites. Wild type allele ‘T’ didn’t show the presence of TFBs.

Known variant

3. A known variant (c.-738A>G) in heterozygous pattern was found in three of 331 cataract cases (2NC and 1PSC) and in one of 210 controls. Wild type allele ‘A’ showed the presence of 2 TFBs, FOXC1 and SOX10 sites. Variant allele ‘G’ showed the presence of binding site for FOXC1 only with a lesser threshold value of 94% and loss of SOX10 site when compared to normal allele ‘A’.

III) IFN-γ +874 A>T polymorphism

1. Considering the genotypic distribution of +874 A>T polymorphism of IFN-γ in female subjects a significant increase in the frequency of AA genotype was observed in general (41.5%) and in different types of cataracts [NC (42.3%); CC (49.4%) and PSC (43.1%)] with corresponding decrease in the frequency of TT (Total: 16.5%; CC: 13.5% and PSC: 9.8%) genotype.

Risk estimates for +874 A>T polymorphism of IFN-γ in different types of cataracts under dominant and recessive model showed a significantly high risk for AA females with CC at 1% (OR=2.3, 95% CI=1.2–4.6; P=0.009), with PSC at 6% (OR=1.8, 95% CI=0.9–3.5; P=0.06) and with NC at 7% level of significance (OR=1.7, 95% CI=0.9–3.4; P=0.07).
Under recessive model, ORs showed significantly high risk for AA and AT individuals for developing PSC (OR=2.9, 95% CI=1.2–7.4; \(P=0.008\)), while for other cataracts the results were insignificant. Females with TT genotype when compared with other two genotypes (AA+AT) among cases and controls showed significant protection for PSC (OR=0.33, 95% CI=0.13-0.83; \(P=0.008\)).

Considering allele frequencies, similar results were obtained showing nearly 2 folds increase in the risk for allele ‘A’ in female patients with CC (OR=1.9, 95% CI=1.2–3.1; \(P=0.004\)) and PSC (OR=1.8, 95% CI=1.1–2.8; \(P=0.004\))

### IV) Expression studies

Expression study was conducted for the mutation p.A199V in exon 7 of \textit{IDO} gene to compare the wild type and mutant proteins. Km values of wild type (68.66±0.26) and mutant IDO proteins (74.92±0.40) \(\mu\text{M}\) showed a significant difference indicating less affinity between the enzyme and substrate for mutant protein compared to wild type.

### CONCLUSIONS

For the first time the present study was carried out to evaluate the contribution of SNP variations detected in all the 10 exons and their intronic regions, and 1.3kb promoter of \textit{IDO} gene in the development of ARCs. Along with this, the association of +874 A>T polymorphism of IFN-\(\gamma\) gene that induces IDO production with ARCs was also studied to evaluate the risk for developing ARC. Apart from this, data from 680 patients and 210 controls were collected on various epidemiological parameters to estimate the risk conferred by them to ARC.

Based on the studies of epidemiological parameters, it was observed that female subjects and subjects above 50 years of age, those with low BMI <25.5 and non-vegetarian food habits and males with the habit of smoking were found to be at greater risk for the development of age-related cataracts.

Logistic regression analysis also supported the influence of age (\(\geq50\) years), female sex, low BMI and non-vegetarian dietary habits as the best independent variables that predict
the onset of the condition. The observations made from logistic regression on the epidemiological parameters suggest that the set of variables that are identified as independent predictors can be used in the prediction of risk a person carries in our population for ARC.

Mutation screening in all the 10 exons with flanking intronic boundaries of *IDO* gene revealed the presence of two exonic novel variants (c.596_597delinsTT in exon 7; C.822C>T in exon 9) in heterozygous state and two intronic known variants (c.422+90 G>A in intron 4; -/CAA deletion in intron 8) registered in NCBI database.

The novel variant c.596_597delinsTT of exon 7 was found in 2 of the cases (1 with NC and 1 with CC), resulting in the substitution of alanine (GCG) to valine (GTT) at position 199 (p.A199V) of amino acid sequence. Polyphen predicted “probable damaging effect” of protein with a significant SIFT score of 0.00. Protein modeling by Triton package also indicated wide variation between the wild type and mutant protein structure. *In vitro* functional studies revealed significant difference in the Km of wild type (68.66±0.26) and mutant IDO proteins (74.92±0.40) μM. It showed lesser affinity between the enzyme and substrate for mutant protein as compared to wild type.

The low affinity between mutant IDO enzyme and substrates is likely to reduce the rate of UV filter synthesis in lens which may be associated with the development of cataract. The low levels of UV filters in lens, makes the lens more vulnerable to exposure of UV light resulting in the cross linking of crystalline proteins present in the lens. Thus the genetic mutation (p.A199V) detected in exon 7 of IDO may be involved in the development of age-related cataracts in the two probands (1 NC and 1 CC) of the present study.

The two variants identified in intronic region (c.422+90 G>A in intron 4 and -/CAA deletion in intron 8) though are not found to affect the potential splice site or branch point directly, they are likely to destruct the enhancer motifs of splicing. Thus these variations may be affecting splicing process leading to alteration of post translational modification of IDO protein. This has to be further confirmed by *in vitro* functional studies.
Mutation screening in 1.3kb promoter of *IDO* gene showed the presence of two novel (c.-979G>A; c.-471T>G) and one known variant (c.-738A>G) in the cataract patients. The creation or destruction of transcription factor binding sites by these variants could possibly be affecting the regulation of IDO expression.

Risk estimates for +874 A>T polymorphism of *IFN-γ* gene that induces *IDO* revealed that female patients with AA genotype were at high risk for developing ARCs specially CC, PSC and NC while TT genotype offered protection for PSC.

The observations made in the present investigations bring out the role of SNP variants in *IDO* gene in the formation of different types of cataracts resulting due to alteration in the structure and function of IDO protein – a vital component required for the normal synthesis and action of UV filters that are responsible to maintain lens transparency and clear vision.

ARC is a multi-factorial condition, which poses public health problem as it is the major cause of blindness in aged individuals. Though surgical extraction of cataractous lenses helps in correcting the defective vision, there are possibilities of the patients suffering from post surgery complications like swelling or edema of the cornea that stalls the perception of normal vision. Hence there is a need to establish exact etiological factors to adopt preventive measures or at least postpone the onset of ARC, so that aged persons can enjoy better quality life with flawless vision. Understanding the molecular genetics of these cataracts is of paramount importance in order to guide the development of therapy that will prevent or delay the cataract onset, and lessens fiscal burden on the affected individuals and also the government. Such attempts will certainly aid to achieve the goal set by the mega approach to prevent blindness-target of vision “2020-right to sight”. Present study is a small contribution made in this direction.