Age-related cataract is a major public health problem leading to blindness in several world populations. They are characterized by the development of opacities in the lens due to several factors that are environmental or genetic in origin. So far about 20 genes have been implicated in the development of ARCs. The present study was conducted to find the association of epidemiological factors and sequence variations in Indoleamine 2,3-dioxygenase (IDO) gene resulting in the development of ARCs apart from estimating the risk caused by IFN-γ +874 A>T polymorphism for ARC formation. The observations made are summarized below

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%; χ²: 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; 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 differed significantly between cases and controls (p≤0.0001).

3. In general 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 found to be 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 for use in the management of ARC.

4. 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. In contrast, males were at risk when they were ≥50 years of age, with low BMI, and with the habit of smoking, and non-vegetarian food intake.

5. Considering frequency distribution of cohorts in different age groups, about 2/3 of cases in age group 50-69 years showed risk for ARCs while the risk was high for female patients in middle age and male patients in older age groups.

6. 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 high risk in females for developing cataracts below 50yrs. In contrast among late onset cases the difference was not significant between the frequencies among females (51.8%) and males (48.2%).

7. 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.

8. 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%; χ²: 4.45; p: 0.03). Among the cohorts studied 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.

9. 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 habit were predicted to be at risk for the development of age-related cataracts.

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

11. Logistic regression analysis also provided evidence for the age ≥50 yrs, 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.

12. Observations made by screening for sequence variations in all 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), and none in exons 1,2-3,5,6 and 10.
13. Screening for variations in exon 7 and intrinsic boundaries showed heterozygous banding pattern in two of the cases (one with NC and one with CC) studied and none among controls. Sequencing of the samples revealed the presence of a novel variation i.e. the deletion and insertion of two nucleotides in succession at position c.596 and c.597 of IDO gene. This is now 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 at position 199 by valine (V). 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. Expression studies conducted for the mutation p.A199V showed significant difference in the Km values of wild type (68.66±0.26) and mutant IDO proteins (74.92±0.40) μM. This indicates low affinity between the enzyme and substrate for mutant protein as compared to wild type protein.

14. Another variant on sequencing of the samples showing mobility shift on SSCP analysis of exon 9 revealed C to T transition in heterozygous condition at c.822 of IDO gene in one of the control subjects. c.822 C>T is a novel synonymous mutation coding for aspartic acid at 274th position of the protein. This variation resulted in loss of restriction site for the enzyme AatII. Use of HSF predicted the break of potential branch point in this variant, and (ESE) and EIE predicted destruction of enhancer site with a score of -100.

15. Screening for variations in the region of exon 4 and its intrinsic boundaries of IDO gene showed mobility shift in 6 patient samples (3 with NC & 3 with PSC). Sequencing of the variant samples revealed the presence of known variation rs4613984 i.e G to A substitution at position c.422+90 in the intron 4. It showed loss of site for HhaI enzyme in variants. Analysis of HSF for the variant c.422+90
G>A predicted the **break of two enhancer sites of splicing** with a score of -100 and creation of new silencer motif with a variation score of -16.23.

16. A known variation rs3214412 showing (-/CAA) deletion in intron 8 was found both among cases and controls of the present study. 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%). **This variation was considered to be a polymorphism.** Estimate of odds ratio showed protection for wild type allele ‘I’ (OR= 0.43; 95%CI =0.18–1.00; P=0.03) in PSC cases. EIEs and PESE octomer from Zhang and Chasin predicted destruction of enhancer site with a variation score of -100.

17. Screening for variations in 1.3kb promoter of *IDO* gene revealed the presence of two **novel variants c.-979 G>A and c.-471 T>G** and one known variant (c.-738 A>G). The presence of variant allele ‘A’ at c.-979 was found in two of the cases (1 with NC & 1 with PSC) resulting in the loss of site for GATA2 transcription factor binding site. This variation created a restriction site for BseMI enzyme.

18. The presence of another novel variation c.-471 T>G in promoter was identified in 3 PSC cases, which created two SPIB and ETS1 transcription factor binding sites.

19. A known variation (c.-738 A>G) in heterozygous pattern was found in 3 of 331 cataract cases (2NC and 1PSC) and one of 210 controls. 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’. The variants in the promoter may be affecting the regulation of *IDO* gene.

20. Considering the genotypic distribution of +874 A>T polymorphism of IFN-γ female subjects showed a significant increase in the frequency of AA genotype when all the types of cataracts were considered (41.5%) and also 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%).

21. 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 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; \( P=0.004 \)).

To conclude the novel sequence variation p.A199V of exon 7 identified in the present study with functional variation can be considered as genetic determinant for ARC. Variants found in promoter sequence affecting TFBs may be involved in the regulation of translation of IDO protein with the possibility of aetiological role in the development of ARCs. The development of novel treatments other than surgery for cataract is currently hindered by incomplete understanding of the processes of cataract formation in lens cells and the underlying cause of this condition at the molecular level. Any investigation made in this direction would be of a great help in exploring measures to prevent or delay the blindness caused by cataract development and hence will have a great role in public health management especially in view of its high prevalence rate in many populations worldwide. It also reduces the fiscal burden on the affected families and the government.