"To Study the Molecular Characterization of X-ray cross-complementing group 1 (XRCC1) gene and its associated Risk factors in Senile Cataract Patients attending a Tertiary Care Centre, Uttar Pradesh."

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Abstract:

Introduction: Ageing of the crystalline lens of the eye, which impairs vision, causes cataracts. Patients who have XRCC1, a DNA repair protein involved in single-strand breaks (SSBs) and the BER pathway, which is in charge of effectively repairing DNA damage, are more likely to develop cataracts.

Aim: To Study the Molecular Characterization with its Special Association to XRCC1 gene in Senile Cataract Patients at a tertiary care centre.

Material and Methods: This was a cross sectional study carried out in the Department of Microbiology & Ophthalmology of RMCH&RC for a period of one year i.e, April 2022 to April 2023. A total of 350 clinical patients were included in which 150 patient were confirmed as cataract positive patients. The 5ml of venous blood was collected in Ethylene diamine tetraacetic acid tubes. The DNA extraction for the detection of XRCC1gene was done using Qiagen DNA Extraction Kit as per manufactures guidelines, which was further confirmed by Rt-PCR.

Results: In the present study the Hypertension 76 (50.6%) was the most common disease associated with the cataract patients. The ratio of Females were more 90 (60%) compared to Males 60 (40%) with the maximum age of 51-60 been affected the most followed by 61-70 years. The other comorbidity included Diabetes). The prevalence of cataract in our study was found to be 42.8%. The presence of XRCC1 gene was detected in all cataract positive patients,which was confirmed by PCR

Conclusion: The polymorphisms of DNA repair genes reduced their capacity to repair DNA damage, rendering the human body significantly more vulnerable to cancer or disorders associated with ageing. The current study's finding that the XRCC1 gene is associated with age-related cataract susceptibility supports the idea that XRCC1 plays a significant role in age-related cataract susceptibility. As a result, early screening and its molecular profiling will aid the clinician in both early diagnosis and early treatment.

Keywords: Cataract, Aging, Crystalline lens, Molecular Profiling, Repair protein, single-strand breaks.

Introduction

Low vision is caused by a cataract, which is an opacification of the lens that blocks light from reaching the retina of the eye. With an estimated 95 million people afflicted worldwide, it is the primary cause of reversible blindness. In India, where vision less than 20/200 in the better eye at presentation is considered blindness, cataract has been shown to be the most significant cause of bilateral blindness [1].

In both industrialised and developing nations, ladies are more likely than males to have cataracts. The onset of cataract is sooner in emerging nations [2]. Compared to western populations, population-based studies have revealed significant incidence rates of cataract in India [3, 4]. There may be significant genetic, environmental, and dietary explanations for these high rates.

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Environmental, nutritional, and genetic factors may be important explanatory factors of these high rates, but till date, there is limited information on these in the Indian setting [5]. The risk factors like diabetes mellitus, high myopia, and occupational exposure to metal work, atopic dermatitis, and smoking were responsible for presenile cataract [6]. The other risk factors include Asthama, thyroid disease and cardiac ailments [7, 8].

DNA repair enzymes continuously monitor chromosomes to correct damaged nucleotide residues Generated by exposure to carcinogens and cytotoxic compounds [9]. Studies have shown that DNA repair gene polymorphisms reduce the ability of these genes to repair DNA damage, rendering the human body much more vulnerable to cancer or age-related disorders [10, 11]. One of the most important DNA repair processes is called base excision repair (BER). Eight-oxoguanine glycosylase-1 (OGG1), AP endonuclease-1 (APE1), and X-ray repair cross-complementing-1 (XRCC1) are the key enzymes of the BER pathway, and associations between gene polymorphisms and age-related macular degeneration, pterygium, and onset primary open-angle

glaucoma have been extensively studied [12,13]. Malignancies, diabetes, and neurological conditions including Huntington's disease are also common alongside ocular problems.

In addition to ophthalmic disorders, malignancies, diabetes and neurological disorders such as Huntington's disease arealso focuses of SNP researches [14, 15].

The proven role of XRCC1 gene plays a very important role in patients associating the risk of cataract, would help the study in understanding the DNA Repair mechanism. Hence, the present study was undertaken to investigate the prevalence, its risk factors and the presence of XRCC1 gene among Senile Cataract patients, as XRCC1 is a DNA repair gene that s emerging as an essential element in the repair of both damaged bases and SSBs marks as an important biomarker of DNA damage.

Material and Methods

This was a cross sectional study carried out in the Department of Microbiology & Ophthalmology of RMCH&RC for a period of one year i.e, April 2022 to April 2023. A total of 350 clinical patients were included. The Demographic details and clinical history along with the relevant clinical investigations like Visual acuity test, Slit-lamp examination, Retinal exam and Applanation tonometry were recorded. Patients affected with cataract and Patients diagnosed as diabetic along with cataract from last 5years or less were included in our study additionally, all the selected study subjects provided their consent to participate in this study. Exclusion criteria for participants involved any patients suffering from any immunocompromised disease and patients who declined their consent to participate in this study like patients with type1 diabetes mellitus, pregnancy and lactating females and also patients with any thyroid disorder, tuberculosis and cancer were excluded from this study. Ethical clearance was taken from the Institutional Ethical Committee of (RMCH&RC).

5ml of venous blood was collected in Ethylene diamine tetraacetic acid tubes. The DNA extraction for the detection of XRCC1gene was done using Qiagen DNA Extraction Kit as per manufactures guidelines, which was further confirmed by PCR.

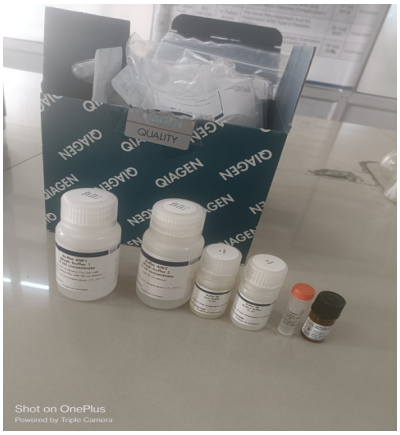
Genotypic method

The Molecular Detection of DNA extraction was done to detect the presence of XRCC1 gene in clinically positive cataract positive patients with the history like personal and demographic data, reason for visit or presenting complaint, past eye history, general medical history, family eye history and allergy history along with examinations like slit lamp examination and applanation tonometry test were recorded.

DNA Extraction: For the detection of XRCC1 gene, chromosomal DNA from the clinical positive cataract patients was done. DNA extraction was carried out using a commercial available the DNA Extraction kit (Qiagen DNA Extraction Kit) as indicated by manufacturer's instructions. Primers used for amplification of XRCC1 gene [16]:

[Table/Fig-1]: The Primer sequence used for the detection of XRCC1 genes

Gene	Primer Sequence (5' to 3')	Size (bp)
XRCC1	F5'-TTGTGCTTTCTCTGTGTCCA-3' R3'-TCCTCCAGCCTTTCTGATA-5'	278 bp



[Table/ Fig-2]: DNA Extraction Kit

[Table/Fig-3]: Primers for XRCC1

Primers was obtained from "Saha gene' and was reconstituted with sterile double distilled water based on the manufacturer's instruction.

Polymerase Chain Reaction (PCR) and its Cycling Conditions:

PCR Cycling:
The PCR conditions were 94°C for 4 minutes, followed by 40 cycles of 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 30 seconds, and a final extension step at 72°C for 10 minutes.

Results

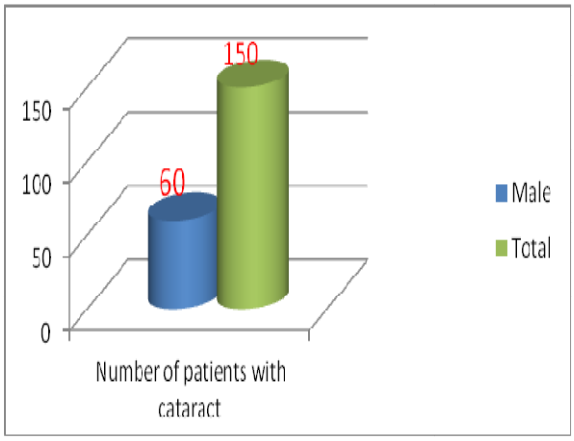
In the present study a total of 350 clinically suspected patients were included in which 150 cases were confirmed as cataract positive patients [Table No.1]. The ratio of Females was more (60%) compared to Males (40%) [Table/Fig-2] with the average age of 51-60 yrs (43.3%) been affected the most followed by 61-70 yrs (23.3%) [Table/Fig-3]. The prevalence of cataract in our study was found to be 42.8%. Hypertension (50.6%) was the most common disease associated with cataract patients.

[Table/Fig-1]: Disease wise Distribution of patients

S. No.	Type of Disease	No. of Isolates	Percentage
1.	Cataract	150	42.8%
2.	Other than Cataract	350	57.2%

[Table/Fig-2]: Gender wise Distribution of Patients with Cataract

S.No.	Gender	Frequency	Percentage
1.	Male	60	40%
2.	Female	90	60 %



[Table/Fig-1]: Gender wise Graphical Representation of Cataract

[Table/Fig-3]: Age wise distribution of Cataract patients

Age wise	No. of Cases	Percentage
40-50	20	13.33%
51-60	65	43.3%
61-70	35	23.3%
71-80	20	13.3%
≥ 81	10	6.6%

[Table/Fig-4]: Disease associated with Cataract patients.

S. No.	Disease	Patients (n= 150)	Percentage
1.	Hypertension	76	50.6 %
2.	Non Hypertensive	11	7.33 %
3.	Diabetes Mellitus	25	16.6 %
4.	Non Diabetic	38	25.3%

Cataract is one of the most common causes of visual impairment in the world. According to the World Health Organization (WHO), cataract is the leading cause of blindness all over the world, responsible for 47.8% of blindness and accounting for 17.7 million blind people [17,18]. In India, 80% of the blindness is due to cataract [19]. Various modifiable risk factors associated with cataract include UV exposure, diabetes, hypertension, body mass index (BMI), drug usage, smoking and socioeconomic factors; but advancing age is the single most important risk factor for cataract.[20-22]. In the present study the Prevalence of cataract was 42.8%. This study was similar to the study conducted by the other author Pajar T. et al., [23] where the prevalence was 34.7%. Other investigators also studied the prevalence where it was found to be 40% [24] and 58%. [17]. There was another study which was in constrict with our study where the prevalence was little lower with 23% [25].

S.No.	Study	Place	Year	Prevalence Rate
1	Present Study	Kanpur	2022	50%
2	Shubhada Sunil Avachat [26]	Maharashtra	2014	53.6%
3	Bourne RRA, et al.[27]	Eastern and Central Europe	2018	44.6%
4	The age-related eye disease study research group [28].	Central Asia	2001	41.6%
5	Present study	RMCH&RC	2023	48.2%

[Table/Fig-5]: The prevalence of Cataract with the other studies

In the present study the ratio of Females was more 90 (60%) compared to Males (40%) with the average age of 61-70 been affected the most and hypertension being the most common disease associated with cataract patients followed by diabetes. This study was in support with the study conducted by Mukharram M et al.,[29] where a higher prevalence of cataract was associated with older age, female sex, urban region of habitation, low level of higher-density lipoproteins, high diastolic blood pressure. In a study conducted by Dhanya V S et al., [30] studied the risk factors accociated with the cataract, where the most common risk factor was diabetes mellitus and hypertension.

The Oxidative stress is supposed to be an important factor in the development of age-related macular degeneration (AMD). XRCC1 gene locates on chromosome 19q13.2. The protein encoded by this gene is involved in the efficient repair of DNA single-strand breaks . Genome instability caused by the great variety of DNA-damaging agents would be an overwhelming problem for cells and organisms if it were not for DNA repair. Base excision repair (BER) is of great importance in DNA excision repair pathway as XRCC1 a key enzyme in base excision repair pathway [31].

In the present study the presence of XRCC1 gene as a DNA repair gene was detected. This finding was parallel to many other studies where XRCC1 gene was detected in senile cataract patients [32-34]. It is noteworthy that XRCC1 was demonstrated to be implicated in single-strand breaks and the BER pathway, which is one of the most important pathways involved in the repair of oxidative and UV-related DNA damage [35]. The association of XRCC1 plays a critical role in the elevated susceptibility to age-related cataracts revealing that this mutation was also regarded as one of the potential mechanisms increasing the risk of age-related cataracts.

Conclusion

As a result of its interactions with DNA ligase III, polymerase beta, and poly (ADP-ribose) polymerase, the XRCC1 protein takes part in the base excision repair pathway. In single-strand break repair, base excision repair, and nucleotide excision repair, XRCC1 is an essential component.

XRCC1 polymorphisms and age-related cataract susceptibility lends credence to the idea that XRCC1 plays a significant role in age-related cataract susceptibility. Therefore, early screening and detection of XRCC1 genetic variants may be helpful for early detection of age-related cataract patients.

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