

Original Research Article

Sanger Sequencing of Exon 14 of RB1 Gene Among North Indian Children with Retinoblastoma

Honey Zahra¹, Sushma Tomar^{*2}, Nikhil Aggarwal³, Nishant Verma⁴, Syed Tasleem Raza⁵, Sanjiv Kumar Gupta⁶, Rakesh Kumar Verma⁷, Punita Manik⁸.

¹ Department of Anatomy, King George's Medical University UP, Lucknow, India.

^{*2} Department of Anatomy, King George's Medical University UP, Lucknow, India.

³ Department of Anatomy, Maulana Azad Medical College, New Delhi, India.

⁴ Department of Paediatrics, King George's Medical University UP, Lucknow, India.

⁵ Department of Biochemistry, Era University, Lucknow, India.

⁶ Department of Ophthalmology, King George's Medical University UP, Lucknow, India.

⁷ Department of Anatomy, King George's Medical University UP, Lucknow, India.

⁸ Department of Anatomy, King George's Medical University UP, Lucknow, India.

ABSTRACT

Introduction: The RB1 gene has been studied for decades. A large number of genetic analytical studies on this gene are published in the Western world, but very few studies from India are available online. RB1 is the most critical gene involved in the causation of retinoblastoma, and Exon 14 is one of the highly mutated exons of this gene.

Aim: To discover the genetic variants in Exon 14 of the RB1 gene in North Indian retinoblastoma patients.

Materials and Methods: This was an inter- and intra-institutional cross-sectional observational study. The Department of Anatomy, Ophthalmology, and Paediatrics at King George's Medical University UP, Lucknow, collaborated with the Department of Biochemistry, ERA University, Lucknow. After obtaining written informed consent from their parents or legal guardians, 40 clinically and radiologically diagnosed retinoblastoma patients were included as study participants. The age range was 12-84 months. 2 ml of peripheral venous blood was withdrawn from 29 patients, and we obtained tumour tissue from 14 patients. We isolated the genomic DNA, which was amplified by PCR, and the amplified products were sent for Sanger sequencing. Chromatograms were analyzed using the BioEdit software.

Results: Most patients (92.5%) were below five years of age. Male preponderance was seen, as there were 37.5% females and 62.5% males. In the majority of cases (72.5%), the tumor was seen unilaterally with predominant involvement of the right eye; while in 27.5% of cases, both eyes were seen affected. We did not observe any variations in the sequences of exon 14.

Conclusions: As the RB1 gene has 27 exons, therefore screening for mutations in all exons is required to reach a concrete conclusion. Genetic variants might be located in the intronic regions, which may affect the proper functioning of the RB1 gene; therefore, sequencing of the entire gene will be more helpful. Next Generation Sequencing (NGS) is preferred because of the large size of the gene.

KEYWORDS: Retinoblastoma, DNA, PCR, RB1 gene, sequencing.

Corresponding Author: Dr. Sushma Tomar, Additional Professor, Department of Anatomy, King George Medical University UP, Shah Mina Road, Chowk, Lucknow, Uttar Pradesh – 226003, India. E-Mail: drsushmatomar@gmail.com

Access this Article online	Journal Information	
Quick Response code  DOI: 10.16965/ijar.2023.279	International Journal of Anatomy and Research ISSN (E) 2321-4287 ISSN (P) 2321-8967 https://www.ijmhr.org/ijar.htm DOI-Prefix: https://dx.doi.org/10.16965/ijar 	
	Article Information	
	Received: 26 Dec 2023 Peer Review: 28 Dec 2023 Revised: 05 Jan 2024	Accepted: 12 Feb 2024 Published (O): 05 Mar 2024 Published (P): 05 Mar 2024

INTRODUCTION

RB1 represents the pioneering tumor suppressor gene, marking the cornerstone of Retinoblastoma research. Its prominence in the context of Retinoblastoma is particularly notable, as this disease predominantly affects children under the age of five. Due to its critical role in human health and pathology, the RB1 gene holds a significant position in the Human Gene Mutation Database (HGMD) [1]. This comprehensive database serves as a repository of diverse mutations discerned within the RB1 gene, complemented by accompanying phenotypic data and pertinent references from the scientific literature. Positioned on chromosome 13q14, the RB1 gene extends across 180 kb and encompasses 27 exons.

The deactivation of the RB1 gene primarily underlies the occurrence of Retinoblastoma, as indicated by GeneReviews [2].

However, emerging studies have suggested the potential involvement of several other genes such as MYCN, CRB1, NUP205, MIR18, NEK7, SOX, and DEK in the intricate development of this disease [3]. Furthermore, epigenetic modifications, including DNA methylation, non-coding RNA-mediated gene silencing, and histone modification-driven chromatin remodeling, have been implicated in the progression of Retinoblastoma. These epigenetic mechanisms serve to suppress or deactivate tumor suppressor genes while concurrently activating oncogenes [4].

The incidence of retinoblastoma has been documented at 1 in every 15000 to 18000 live births [3,5]. Globally, approximately 5000 new cases are reported annually, with India alone contributing 1500 to 2000 cases. The mortality rate associated with retinoblastoma is notably higher in Asian and African countries (20-60%) when compared to that in developed nations (3-5%). Several significant factors contributing to this increased mortality include delayed diagnosis, advanced disease presentation, limited access to advanced medical services, and inadequate management protocols within these countries [6].

Retinoblastoma manifests in two distinct forms: non-heritable and heritable. Non-heritable retinoblastoma typically arises from somatic mutations affecting both alleles of the RB1 gene. On the other hand, heritable retinoblastoma results from the inheritance of at least one germline mutation, combined with an acquired somatic mutation in the RB1 gene. In most cases, heritable retinoblastomas are observed bilaterally, while non-heritable cases tend to be unilateral. Moreover, the offspring of individuals affected by hereditary retinoblastoma are at an increased risk of developing the disease, as the trait is transmitted in an autosomal dominant manner, with a penetrance of 80% to 90% [1].

The genetic testing of retinoblastoma patients can be carried out using several methods such as Polymerase Chain Reaction (PCR), Sanger sequencing, Multiplex Ligation-dependent Probe Amplification (MLPA), and Next-generation sequencing (NGS) [7].

Affected children typically undergo a series of clinical evaluations, including examination under anesthesia (EUA), to enable early detection and treatment of tumors. Early identification of carriers with heritable RB1 mutations and timely diagnosis can significantly enhance disease management and overall outcomes [1]. While the prognosis of retinoblastoma is generally favorable with early detection, delayed diagnosis can potentially pose life-threatening risks [8].

The RB1 gene has been a subject of study for several decades, with a substantial body of research emanating from the Western world, while a limited number of studies are accessible online from India [9,10].

Notably, research on the RB1 gene concerning retinoblastoma has been predominantly concentrated in the Southern Indian region, as far as current knowledge permits [11].

Furthermore, the RB1 gene is recognized for its distribution of CpG islands spanning various exons, including exon 8, 10, 11, 14, 15, 17, 18, and 23, which serve as notable mutational hotspots. Extensive literature attests to the occurrence of mutations across all exons of the RB1 gene [1].

Given that exon 14 falls within this range of mutational hotspots, our research endeavours aim to uncover the genetic variants within Exon 14 of the RB1 gene in North Indian patients diagnosed with retinoblastoma.

MATERIALS AND METHODS

Study setting: The present study was done intra- and inter-institutional collaboration. The Department of Anatomy, Ophthalmology, and Paediatrics at King George's Medical University UP, Lucknow, collaborated with the Department of Biochemistry at Era University, Lucknow, India.

Study participants: All cases of Retinoblastoma who attended the Outpatient Department (OPD) and Inpatient Department (IPD) of the Department of Ophthalmology, KGMU UP, Lucknow; from February 2021 to January 2022 were screened for inclusion and exclusion criteria. After exclusion, we got 40 study subjects (Age range: 12-84 months, Mean age: 38.23 months). Out of 40, 25 were male and 15 were female.

Sample collection: 2ml of peripheral venous blood was withdrawn from 29 unrelated study subjects in an EDTA vacutainer. We were not able to get blood samples of 11 patients (8 males and 3 females), the reason being either they were on chemotherapy or their operative procedure was canceled. We also obtained fresh tumor tissue in a container having normal saline from 14 patients who had undergone enucleation. We stored all the samples at -20 °C.

Ethical considerations: This study was approved by the Institutional Ethics Committee (IEC) of King George's Medical University UP, Lucknow (Ref. Code: IV PGTSC-IIA/P32). Legal guardians of all study subjects provided informed consent to perform clinical tests and anonymously use the samples for medical research.

DNA Isolation: Genomic DNA was isolated from blood and tumor tissue using Nucleospin Plasmid Quick Pure Kit (manufactured by Macherey–Nagel) in the Central research laboratory, Era University, Lucknow. Genomic

DNA extraction was confirmed by agarose gel electrophoresis, and the DNA was stored at -20 °C until use.

Quality Check of isolated DNA: The quality of isolated DNA was checked on Nano Drop™2000/2000c Spectrophotometer (Manufactured by ThermoScientific™) (**Figure 1**). The maximum absorbance of nucleic acid is 260nm to measure the purity of DNA. The ratio of this absorbance maximum to the absorbance at 280 nm has been used. A 260/280 ratio of ~1.8 is commonly accepted as “pure” for DNA. Abnormal 260/280 ratios usually show that a sample is contaminated by residual phenol, guanidine, or other reagents used in the extraction protocol, in which case the ratio is usually low. For this, we took 2µL of DNA with 0-50 µl pipette.

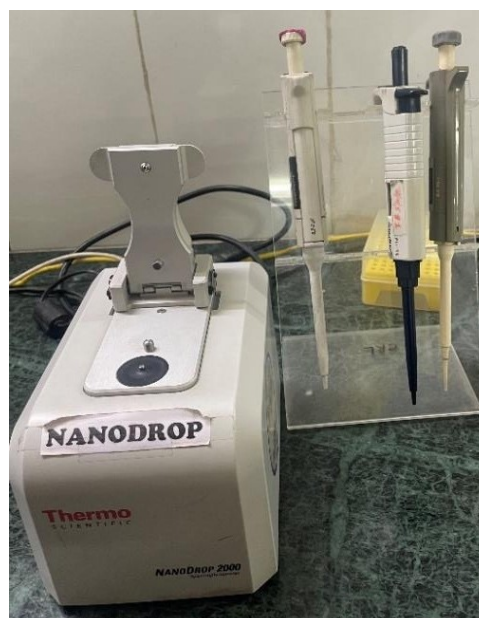


Fig.1: NanoDrop™2000/2000c Spectrophotometer[(Manufactured by ThermoScientific™). Source: Central Research Laboratory, Department of Biotechnology, Era University, Lucknow.]

PCR amplification: The isolated DNA had been amplified by Polymerase Chain Reaction (PCR) using forward and reverse primers for Exon 14 of the RB1 gene. The primers were synthesized by Eurofins Genomics India Pvt. Ltd.#540/1, Doddanakundi Industrial Area 2, Hoodi, Whitefield, Bengaluru, Karnataka, 560048. India. The sequences of primers were taken from a research article by Braggio E et al. [12]. The primers were stored at -20 °C.

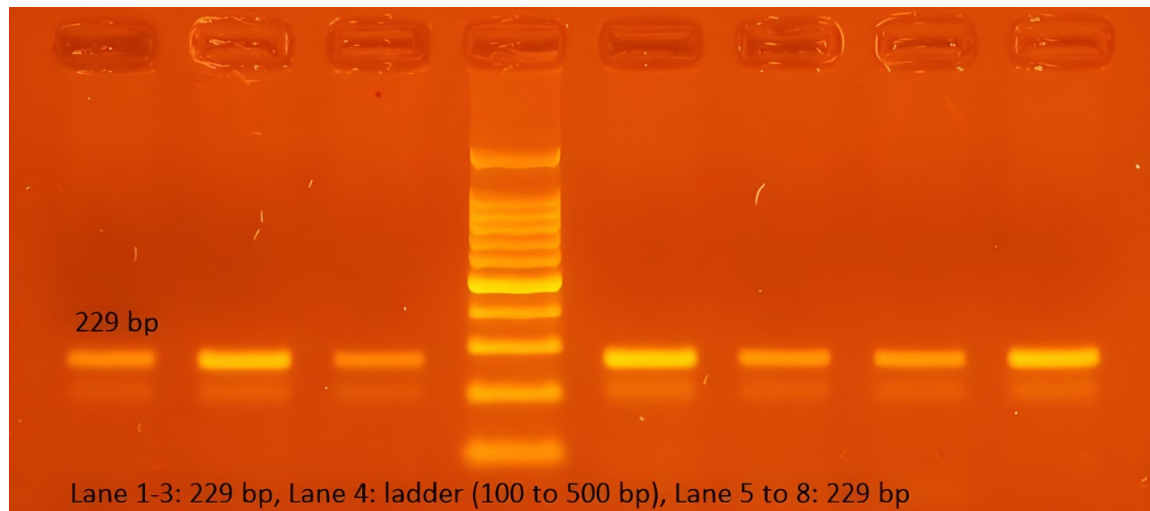
Primers: The sequence of primers and other parameters are depicted in **Table 1**.

Table 1: Primers for Exon 14 of RB1 gene.

Exon	Primer	Sequence (5'-3')	Length	M P* [Tm](°C)	GC** (%)
14	Forward	ATTGTGATTTTCTAAAATAGCAGG	24	54.18	29.17
	Reverse	CAGGATGATCTTGATGCCTTG	21	57.87	47.62

*MP-Melting Point

**GC- Guanine Cytosine

**Fig. 2:** Gel picture of Exon 14 bp.

The PCR had a total volume of **20 µl**, including **10 µl** Master mix, **6 µl** nuclease-free water, **1 µl** forward and reverse primers each, **2 µl** DNA, **0.1µl** MgCl₂[ABI] (25 mili Mole), and **0.1µl** Taq Polymerase. We performed the PCR on Thermocycler (manufactured by Bio-Rad T100™ Thermal Cycler). The Thermocycler conditions for PCR of Exon 14 of RB1 gene were as follows: **initial denaturation** at **94 °C** for **2 min** followed by **40 cycles** of **denaturation** at **94 °C** for **30 sec**, **annealing** at **52.5 °C** for **30 sec**, and **extension** at **72 °C** for **30 sec**. Final extension is given at **72 °C** for **2 min**. At a temperature of **72 °C**, DNA polymerase acts well, synthesizing the DNA strands by sequentially adding nucleotides to the primers, utilizing the target DNA as a template. Amplified PCR products were run on the agarose gel, and the image was taken from the Gel Doc (Figure 2).

Sanger Sequencing: Sanger sequencing was performed by Eurofins Genomics India Pvt. Ltd for the amplified PCR products. They performed the sequencing on a Sequencer DNA analyser 3730XL 96 capillary array, manufactured by Thermofisher.

We received the sequencing data in chromatograms or traces (.ab1 files) via email in a Zipped folder.

Analysis and Interpretation of Sequences: We analyzed the chromatograms on a computer system with the help of BioEdit software. To identify the heterozygous variants, we looked at double peaks, and the MegaBLAST tool (NCBI website) was used for homozygous variants. If we found any sequence variation on the forward sequencing file, we also looked for the same variant in the reverse sequencing file to confirm that. If any variation had been observed either in the forward or reverse sequencing file only, then we considered it as an artefact.

Various tools and databases (ClinVar, gnomAD, VarSome, Franklin, etc.) were used for the mutational analysis.

Statistical Analysis: It was performed by using statistical package for social sciences (SPSS) software. The continuous variables were evaluated by mean or range value when required. The dichotomous variables were presented in numbers and were analyzed by using the Chi-square test. A p-value of <0.05 or 0.001 was regarded as significant.

RESULTS

The mean age of the study participants was 38.23 ± 17.14 months (Age range: 12 – 84 months). 32.5% of study subjects were between the ages of 37 and 60 months. Only 3 (7.5%) study participants were >60 months

Table 2: Age-wise Distribution of Study Subjects.

S. No.	Age (months)	Age (years)	Number of Study Subjects (N)	% of Study Subjects
1	12-24	1-2 years	12	30
2	>24-36	>2-3 years	12	30
3	>36-60	>3-5 years	13	32.5
4	>60	>5 years	3	7.5
Total			40	100

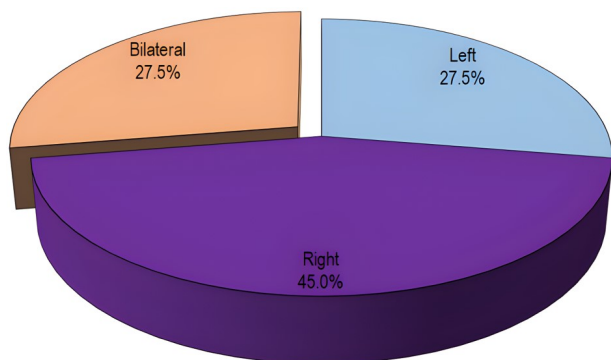
of age. Rest 60 % were equally distributed among age groups 12-24 months and >24-36 months (30.0% each) [Table 2].

The majority of the study subjects were males (62.5%), and the rest were females (37.5%) (Table 3).

Table 3: Gender-wise Distribution of Study Subjects.

S. No.	Gender	Number (N)	%
1	Female	15	37.5
2	Male	25	62.5
Total		40	100

The majority of the study participants presented with unilateral retinoblastoma. Out of 40, unilateral retinoblastoma was observed in 29 (72.5%), whereas 11 (27.5%) participants had a tumor in both eyes. Among these cases of retinoblastoma, the right eye was involved in 18 (45%), and the left eye was involved in 11 (27.5%) cases, together constituting 72.5% of total unilateral cases (Figure 3). The gender-wise frequency distribution of bilateral and unilateral cases was similar (Table 4).

**Fig. 3:** Pie Diagram showing Laterality wise Distribution of Study Subjects.

During history taking, we asked the parents/ legal guardians of the patients for the age at diagnosis of retinoblastoma. Although we had 11 cases of bilateral retinoblastoma, we were able to retrieve the information only in 8 patients. Among 29 cases with unilateral retinoblastoma, we could retrieve the information only in 11 patients (Table 5).

By this comparison, we inferred that the diagnosis of retinoblastoma in Children with bilateral presentation was made at an earlier age while the children with unilateral retinoblastoma were diagnosed late. This difference was statistically significant ($p < 0.001$).

We did not observe any variants in the sequences of Exon14. All the forward and reverse sequences of exon 14 were fully matched with the reference sequence of exon 14 of *RB1* gene taken from the Ensembl genome browser 108 in GRCh 37 genome assembly (Figure 4) (Transcript ID- ENST00000267163.4) which is as follows:

CGATACAACTTGGAGTTCGCTTGATTACCG AGTAATGGAATCCATGCTTAAATCA [57 bases; Co-ordinates: 48953730-48953786]

Table 5: Comparison between Bilateral and Unilateral Cases of Retinoblastoma with regards to Age at Diagnosis.

S. No. of B/L cases of Retinoblastoma	Age at Diagnosis (months)	S. No. of U/L cases of Retinoblastoma	Age at Diagnosis (months)
1	7	1	36
2	8	2	36
3	9	3	48
4	9	4	48
5	24	5	48
6	24	6	48
7	30	7	48
8	36	8	60
Age Range	7-36	9	60
Mean Age \pm SD (months)	18.38 \pm 11.48	10	60
		11	60
		Age Range	36-60
		Mean Age \pm SD (months)	50.18 \pm 9.01

$t' = 6.779$; $p < 0.001$

Table 4: Association between Laterality of Retinoblastoma and Gender of Study Subjects.

Laterality of Retinoblastoma	Study Subjects		Female Subjects		Male Subjects	
	N	%	N	%	N	%
Unilateral	29	72.5	11	73.3	18	72
Bilateral	11	27.5	4	26.7	7	28
Total	40	100	15	100	25	100

$\chi^2 = 0.008$; $p = 0.927$

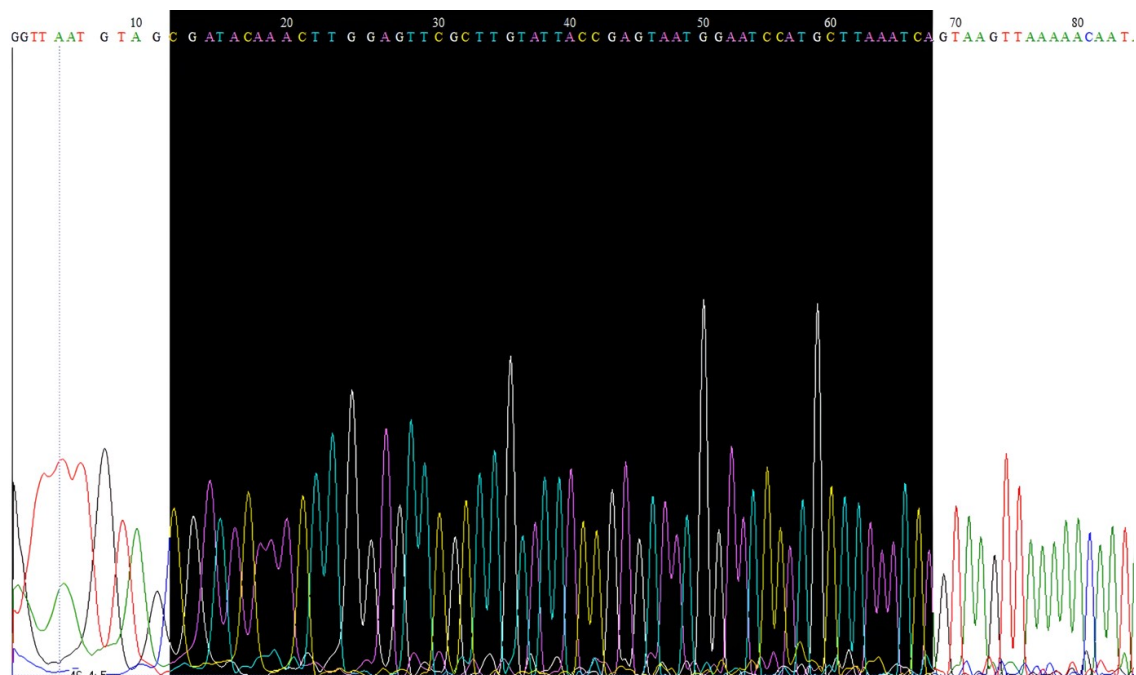


Fig. 4: Fully matched forward sequence of Exon 14 with Reference Sequence.

DISCUSSION

Retinoblastoma, a malignant eye tumor primarily affecting children under the age of five years [13], originates from the nuclear layers of the retina [14]. Apart from creating sight problems, this tumor can also be potentially life-threatening [14].

The biallelic inactivation of the RB1 gene is a known cause of retinoblastoma [1], as RB1 regulates various cell cycle components. Additionally, the occurrence of this tumor is influenced not only by genetic changes but also by epigenetic alterations [15]. The age at diagnosis of retinoblastoma is not only affected by the laterality of the disease but also by socio-economic factors, as indicated by a study conducted by Kumar A et al, highlighting late presentations in developing countries [16].

Notably, the average age of our study subjects was 38.23 ± 17.14 months, which closely aligns with the age range (34.7 ± 24.6 months) documented in a related study conducted in India [15], where 618 eyes from 467 patients were examined. This observed age range aligns with countries categorized under the medium Human Development Index (HDI). HDI categorizes countries into four groups based on education, income per capita, and life expectancy statistics, namely very high, high, medium, and poor. Notably, in countries with

very high HDI, the average age of presentation is typically less than one year [14].

However, our study indicated an older age at the time of diagnosis, attributed to the lower socioeconomic strata and predominantly rural population of the subjects. Within our study cohort, we observed delays in diagnosis of up to 84 months. Given that India falls within the range of medium HDI countries, the lack of awareness and education may contribute to a lag between the onset of symptoms and diagnosis [17].

Within our study, most subjects diagnosed with retinoblastoma were male (62.5%), resulting in a male-to-female ratio of 1.67. In contrast, a study conducted by Ibrahim NO et al. demonstrated a female preponderance, with a male-to-female (M/F) ratio of 0.7, which was statistically insignificant. Notably, none of the subjects in our study had a positive family history of retinoblastoma. Our findings align with gender distributions reported from various countries, including Mexico (52.4%), Mali (54.5%), Egypt (60.25%), and Jordan (70.0%) [18,19], indicating a similar male predominance. It is worth noting that research in our nation has exhibited a discernible regional bias, possibly due to the historical preference for treating men in prior studies [15]. Moreover, the male-to-female (M/F) ratio in the

Table 6: Comparative Analysis of Published Literature on Genetic Testing of RB1 Gene in Retinoblastoma Patients.

Authors & Year of Study	Study Population & Sample Size	Genetic Test	Laterality of Retinoblastoma	Analyzed regions of RB1 Gene	Variants Detected
Parsam, V.L. <i>et al.</i> , 2011 [9]	Indian, 74	Quantitative Fluorescence-based Multiplex Polymerase Chain Reaction, Fluorescent Genotyping of RB1 Alleles, Polymerase Chain Reaction-Restriction Fragment Length Polymorphism, Sequencing	53 Bilateral & 21 Unilateral	Promoter region & exons	Large deletions, small deletions/insertions, point mutations, nonsense, splice site, substitution, silent mutation
Kiran <i>et al.</i> , 2003 [10]	Indian, 47	Sequencing, Single-Strand Conformation Polymorphism	32 Bilateral 15 Unilateral	Promotor region & exons	Nonsense & frameshifts, single base changes, small deletion, duplication
Alonso J <i>et al.</i> , 2001 [22]	Spanish, 43	Direct Polymerase Chain Reaction Sequencing	NA	Exon 2-27	Nonsense, frameshifts & splicing mutations
Abidi O <i>et al.</i> , 2011 [23]	Moroccan, 41	Polymerase Chain Reaction, DNA Sequencing	25 Bilateral 16 Unilateral	Promoter region & exons	Nonsense/frameshift, single base substitution, splice site mutation
Nguyen H.H. <i>et al.</i> , 2018 [24]	Vietnamese, 34	Polymerase Chain Reaction, DNA Sequencing	25 Bilateral 9 Unilateral	Flanking and promoter region, exon region	Nonsense /frameshift mutations, whole gene deletion, missense mutations
Present Study 2022	Indian, 40	Polymerase Chain Reaction, DNA Sequencing	11 Bilateral 29 Unilateral	Exon 14 region	No Mutation /Single Nucleotide Polymorphisms detected

Population-Based Cancer Registries (PBCRs) in Bangalore (2.0) and Chennai (1.9) was significantly higher compared to other PBCRs. Conversely, research from major cities in India, such as Chennai and Mumbai, has reported M/F ratios of 1.0 and 1.4, respectively [20]. Several researchers in the Indian context have also reported male predominance, with Padma *et al.* (2020) observing a male-to-female ratio of 1.2:1 in the Indian study population [11].

In a study encompassing 600 retinoblastoma patients, it was observed that the tumor manifested solely in one eye in 67.6% of cases, whereas in 32.4% of cases presented with the tumor in both eyes [6]. Similarly, Gupta *et al.* reported that a significant proportion (70–75%) of retinoblastoma cases in their study were unilateral [13]. Another study conducted in India by Singh *et al.* also exhibited a preponderance of instances of unilateral retinoblastoma (67.6%) compared to cases with bilateral involvement (32.3%) [15]. Within our present study, we identified bilateral retinoblastoma in 27.5% of the study subjects, while 72.5% of participants exhibited unilateral tumor manifestation. Notably, the right eye was affected in most (45%) unilateral cases, while the remaining 27.5% involved the left eye. When considering the association between gender and the laterality of retinoblastoma, we found that both

genders demonstrated a higher number of unilateral cases than bilateral retinoblastoma, with a similar frequency observed in females (73.3%) and males (72.0%). The incidence of bilateral retinoblastoma was also comparable between males (28.0%) and females (26.7%). As a result, our comparison of the laterality of retinoblastoma between male and female study participants did not reveal any statistically significant differences.

In a study published in GeneReviews by Lohmann DR and Gallie BL (2000), it was revealed that approximately 60% of affected individuals exhibit unilateral retinoblastoma, with a mean age of diagnosis recorded at 24 months, while about 40% present with bilateral retinoblastoma, with a mean age of diagnosis reported at 15 months [2]. Similarly, our study also identified a statistically significant difference ($p < 0.001$) in the mean age at diagnosis between unilateral and bilateral cases, a finding that was also observed by Bonanomi *et al* [14].

Considered one of the worldwide reported hotspots in a searchable database, mutations in exon 14 have garnered significant attention [11,19–22]. Through the utilization of several computational approaches such as SIFT, PolyPhen-2, I-mutant, and Project Hope, reported mutations worldwide have been identified, including two significant non-synonymous single nucleotide polymorphisms

(nsSNPs) (CDNA 1333C>T, protein R445X, and CDNA 1363C>T, R455X), which are believed to contribute to the malfunction of the native RB1 protein, potentially leading to carcinoma [5]. However, in our study, these targeted mutations were not detected in exon 14 of the RB1 gene within the North Indian population. As mutations in the RB1 gene are known to occur randomly and may be located in various exons due to the heterogeneity of the disease, a comprehensive screening of all exons would be more appropriate. Furthermore, our findings are consistent with several previous studies from different countries, which also revealed diverse mutations across the RB1 gene, albeit not specifically within exon 14, among patients with retinoblastoma (including cases of unilateral, bilateral, sporadic, and familial occurrences) [23].

A comparative analysis of this published literature on genetic testing of RB1 gene in retinoblastoma patients is given in **Table 6** for reference.

CONCLUSION

The present study emphasizes the impact of socioeconomic factors on the age of diagnosis, gender-specific prevalence patterns, and the prevalent unilateral presentation of retinoblastoma. While not detecting specific mutations in exon 14 of the RB1 gene within the North Indian population, our findings reiterate the need for comprehensive screening across all exons, considering the random occurrence and heterogeneity of the disease. Thus, this study contributes to the global understanding of retinoblastoma and calls for continued research into its complex genetic and socioeconomic underpinnings for improved diagnostic and therapeutic interventions.

Author-Co-Author Contributions to the manuscript

HZ: Design of study, Performed the experiments, Literature survey, Implementation of study protocol, Data analysis, Manuscript preparation

ST: Designed and conceptualised the study, Implementation of study protocol, Data analysis, Reviewed the results, Manuscript

preparation.

NA: Coordination and Manuscript preparation, Revision, Language editing and Submission of an article

NV: Coordination and Manuscript review and revision

STR: Implementation of study protocol and Manuscript preparation.

SKG: Guidance in Manuscript preparation and Reviewed the manuscript

RKV: Guidance in Manuscript preparation and Reviewed the manuscript

PM: Reviewed the Manuscript and Manuscript revision

ORCID

Honey Zahra- 0009-0002-0343-3621

Sushma Tomar- 0000-0002-9088-3171

Nikhil Aggarwal- 0000-0002-4653-7060

Nishant Verma- 0000-0002-8997-1801

Syed Tasleem Raza- 0000-0003-1248-8974

Sanjiv Kumar Gupta- 0000-0002-8543-2915

Rakesh Kumar Verma- 0000-0002-8984-7587

Punita Manik- 0000-0001-9942-250X

Conflicts of Interests: None

REFERENCES

- [1]. Tomar S, Sethi R, Sundar G, Quah TC, Quah BL, Lai PS. Mutation spectrum of RB1 mutations in retinoblastoma cases from Singapore with implications for genetic management and counselling. PLoS One 2017;12. <https://doi.org/10.1371/journal.pone.0178776> PMID:28575107 PMCID:PMC5456385
- [2]. Lohmann DR, Gallie BL. Retinoblastoma. GeneReviews® 2023.
- [3]. Rushlow DE, Mol BM, Kennett JY, Yee S, Pajovic S, Thériault BL, et al. Characterisation of retinoblastomas without RB1 mutations: genomic, gene expression, and clinical studies. Lancet Oncol 2013;14:327-34. [https://doi.org/10.1016/S1470-2045\(13\)70045-7](https://doi.org/10.1016/S1470-2045(13)70045-7) PMID:23498719
- [4]. Singh U, Malik MA, Goswami S, Shukla S, Kaur J. Epigenetic regulation of human retinoblastoma. Tumour Biol 2016;37:14427-41. <https://doi.org/10.1007/s13277-016-5308-3> PMID:27639385
- [5]. Valverde JR, Alonso J, Palacios I, Pestaña Á. RB1 gene mutation up-date, a meta-analysis based on 932 reported mutations available in a searchable database. BMC Genet 2005;6. <https://doi.org/10.1186/1471-2156-6-53> PMID:16269091 PMCID:PMC1298292

- [6]. Chawla B, Singh R. Recent advances and challenges in the management of retinoblastoma. *Indian J Ophthalmol* 2017;65:133.
https://doi.org/10.4103/ijo.IJO_883_16
PMid:28345569 PMCID:PMC5381292
- [7]. Markovič L, Bukovac A, Varošane AM, Šlaus N, Pečina-Šlaus N. Genetics in ophthalmology: molecular blueprints of retinoblastoma. *Hum Genomics* 2023;17:1-15.
<https://doi.org/10.1186/s40246-023-00529-w>
PMid:37658463 PMCID:PMC10474694
- [8]. Ibrahim NO, Saleem M, Eltayeb E, Mekki S, Elnaim EG, Idris AB, et al. Molecular Genetic Analysis of RB1 gene among Sudanese children with Retinoblastoma. *BioRxiv* 2019:519306.
<https://doi.org/10.1101/519306>
- [9]. Parsam VL, Ali MJ, Honavar SG, Vemuganti GK, Kannabiran C. Splicing aberrations caused by constitutional RB1 gene mutations in retinoblastoma. *J Biosci* 2011;36:281-7.
<https://doi.org/10.1007/s12038-011-9062-9>
PMid:21654082
- [10]. Kiran VS, Kannabiran C, Chakravarthi K, Vemuganti GK, Honavar SG. Mutational screening of the RB1 gene in Indian patients with retinoblastoma reveals eight novel and several recurrent mutations. *Hum Mutat* 2003;22:339. PMid:12955724
<https://doi.org/10.1002/humu.9181>
- [11]. Padma M, Kumar N, Nesargi P, Aruna Kumari B, Appaji L, Viswanathan A. Epidemiology and clinical features of retinoblastoma: A tertiary care center's experience in India. *South Asian J Cancer* 2020;9:56-8.
https://doi.org/10.4103/sajc.sajc_89_19
PMid:31956625 PMCID:PMC6956589
- [12]. Braggio E, Bonvicino CR, Vargas FR, Ferman S, Eisenberg ALA, Seuánez HN. Identification of three novel RB1 mutations in Brazilian patients with retinoblastoma by "exon by exon" PCR mediated SSCP analysis. *J Clin Pathol* 2004;57:585.
<https://doi.org/10.1136/jcp.2003.014423>
PMid:15166261 PMCID:PMC1770321
- [13]. Gupta H, Malaichamy S, Mallipatna A, Murugan S, Jeyabalan N, Suresh Babu V, et al. Retinoblastoma genetics screening and clinical management. *BMC Med Genomics* 2021;14.
<https://doi.org/10.1186/s12920-021-01034-6>
PMid:34294096 PMCID:PMC8296631
- [14]. Bonanomi MTBC, de Almeida MTA, Cristofani LM, Odone Filho V. Retinoblastoma: A Three-Year-Study at a Brazilian Medical School Hospital. *Clinics (Sao Paulo)* 2009;64:427.
<https://doi.org/10.1590/S1807-59322009000500010>
PMid:19488609 PMCID:PMC2694247
- [15]. Singh U, Katoch D, Kaur S, Dogra MR, Bansal D, Kapoor R. Retinoblastoma: A Sixteen-Year Review of the Presentation, Treatment, and Outcome from a Tertiary Care Institute in Northern India. *Ocul Oncol Pathol* 2017;4:23-32.
<https://doi.org/10.1159/000477408>
PMid:29344495 PMCID:PMC575756
- [16]. Kumar A, Moulik NR, Mishra RK, Kumar D. Causes, outcome and prevention of abandonment in retinoblastoma in India. *Pediatr Blood Cancer* 2013;60:771-5.
<https://doi.org/10.1002/pbc.24454>
PMid:23303533
- [17]. Dimaras H, Kimani K, Dimba EAO, Gronsdahl P, White A, Chan HSL, et al. Retinoblastoma. *The Lancet* 2012;379:1436-46.
[https://doi.org/10.1016/S0140-6736\(11\)61137-9](https://doi.org/10.1016/S0140-6736(11)61137-9)
PMid:22414599
- [18]. Boubacar T, Fatou S, Fousseyni T, Mariam S, Fatoumata DT, Toumani S, et al. A 30-month prospective study on the treatment of retinoblastoma in the Gabriel Toure Teaching Hospital, Bamako, Mali. *Br J Ophthalmol* 2010;94:467-9.
<https://doi.org/10.1136/bjo.2009.159699>
PMid:19822911
- [19]. Leal-Leal C, Flores-Rojó M, Medina-Sansón A, Cerecedo-Díaz F, Sánchez-Félix S, González-Ramella O, et al. A multicentre report from the Mexican Retinoblastoma Group. *Br J Ophthalmol* 2004;88:1074-7.
<https://doi.org/10.1136/bjo.2003.035642>
PMid:15258028 PMCID:PMC1772266
- [20]. Yeole BB, Advani S. Retinoblastoma: An Epidemiological Appraisal with Reference to a Population in Mumbai, India. *Asian Pac J Cancer Prev* 2002;3:17-21.
- [21]. Lan X, Xu W, Tang X, Ye H, Song X, Lin L, et al. Spectrum of RB1 Germline Mutations and Clinical Features in Unrelated Chinese Patients With Retinoblastoma. *Front Genet* 2020;11.
<https://doi.org/10.3389/fgene.2020.00142>
PMid:32218800 PMCID:PMC7080181
- [22]. Alonso J, Garca-Miguel P, Abelairas J, Mendiola M, Sarret E, Teresa Vendrell M, et al. Spectrum of germline RB1 gene mutations in Spanish retinoblastoma patients: Phenotypic and molecular epidemiological implications. *Hum Mutat* 2001;17:412-22. PMid:11317357
<https://doi.org/10.1002/humu.1117>
- [23]. Abidi O, Knari S, Sefri H, Charif M, Senechal A, Hamel C, et al. Mutational analysis of the RB1 gene in Moroccan patients with retinoblastoma. *Mol Vis* 2011;17:3541.
- [24]. Nguyen HH, Nguyen HTT, Vu NP, Le QT, Pham CM, Huyen TT, Manh H, Pham HLB, Nguyen TD, Le HTT, Van Nong H. Mutational screening of germline RB1 gene in Vietnamese patients with retinoblastoma reveals three novel mutations. *Mol Vis*. 2018 Mar 17;24:231-238.
PMID: 29568217; PMCID: PMC5858657.

How to cite this article: Sanger Sequencing of Exon 14 of RB1 Gene Among North Indian Children With Retinoblastoma. *Int J Anat Res* 2024;12(1):8862-8870. DOI: 10.16965/ijar.2023.279