Clinicopathological Features and RB1 Gene Polymorphism in Sudanese Retinoblastoma Patients

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Abstract: *Background*: Retinoblastoma is a highly aggressive eye cancer affecting infants and young children, with outcomes dependent on early diagnosis. Mortality remains high in low-resource settings. This study aimed to characterize retinoblastoma among Sudanese patients, including an analysis of clinicopathological features and the status of RB1 gene polymorphisms.

Material and Methods: This is cross-sectional study included 99 Sudanese patients diagnosed with retinoblastoma in Khartoum Teaching Eye Hospital and Mecca Eye Complex. Clinicopathological features and demographic data were retrieved from patient's medical records and histopathology Laboratory Information System (LIS). Genomic DNA was isolated from formalin fixed paraffin embedded (FFPE) retinoblastoma tissue blocks. Detection of RB 1 gene polymorphism was conducted through Polymerase Chain Reaction (PCR). BLAT search genome used to compare the results of breakpoint sequencing with a reference genomic sequence.

Results: Total of 99 retinoblastoma patients were included, (53.5%) were female and (46.5%) were male, with (79.8%) having unilateral lesions and (20.2%) bilateral. Nerve invasion was present in (44.4%) of the cases. Half of patients were diagnosed at advance stage (T4) (58.6%) and at high grade were (Grade 3) (69.7%). (15%) of the cases were from Damazin state, followed by Nyala (13.1%).

Conclusion: Detection of retinoblastoma in Sudanese patients often occurs at advanced stages and grades, leading to poorer clinical outcomes. Genetic testing is crucial to identify individuals predisposed to this illness, enabling early intervention and management.

Keywords: Retinoblastoma, RB1 gene, Sudanese patients, Clinicopathological features, Genetic polymorphism.

1. INTRODUCTION

Retinoblastoma (RB) is the most common intraocular tumor among children, accounting for around 3% of all pediatric tumors. Retinoblastoma affects one in every 20,000 newborns. Early diagnosis leads to a favorable prognosis, but late stages result in a poor clinical outcome. The most common signs of RB are leukocoria and strabismus [1]. A recent study aimed to assess the pattern of pediatric cancer in Khartoum Oncology Hospital, the main hospital that treats cancer patients across the nation in Sudan, located in the capital city, Khartoum. Results showed that retinoblastoma was the fourth most common malignancy among Sudanese children. Around 410 cases were diagnosed during a period of 15 years; event-free survival was 52% for children with retinoblastoma [2]. Another earlier report (2011) showed that the retinoblastoma count is around 4.8% of pediatric cancer cases in Sudan. The report states that out of 25 children diagnosed with retinoblastoma at the Al-Gezira Oncology Centre, 11 were suffering from metastatic disease, while 5 had regional disease and localized illness in 9 patients. 22 eyes were enucleated (ten unilateral and six bilateral) [2].

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Retinoblastoma appears as a hereditary (40%) and nonhereditary disease (60%) [3]. When it is present in its hereditary form, it is inherited as an autosomal dominant trait. The responsible gene attributed to this tumor is the retinoblastoma susceptibility gene (RB1), which is located in chromosome 13q14 [4]. RB1 is proposed to undergo inactivation during the development of the majority of cancer types [5]. RB1, the tumor suppressor, is part of cellular pathways essential for regulating the transition from the G1 to the S phase in the cell cycle and responding to various external and internal signals. In more detail, the encoded RB protein and its belonging family members (p107 and p130) are components of a signaling pathway that maintains the cellular response to various signals by controlling the activity of the transcription factor (E2F) and the expression of E2F target genes during the progression of the cell cycle. In normal physiology, RB1 is suppressed by cyclin-dependent kinase (CDK) in response to growth signals. When the RB1 gene is upregulated, mutated, or its promoter is methylated, the function of RB1 is impaired; consequently, the gene loses its capacity to regulate E2F transcription factor, potentially contributing to cancer initiation and progression [.]. Although polymorphisms in RB1 were well established earlier in retinoblastoma and other malignancies [7-10], However, information reflecting the RB1 polymorphism status among Sudanese retinoblastoma patients is limited. Previous research has shown that the frequency and presence of these alleles differ significantly across various ethnic groups [4].

In the present study, we aimed to assess clinico-pathological features and RB1 gene polymorphisms in Sudanese children with retinoblastoma.

2. MATERIAL AND METHODS

2.1. Retinoblastoma Patients

This cross-sectional study was conducted at Khartoum Teaching Eye Hospital and Mecca Eye Complex, both located in Khartoum State, Sudan, between January 2019 and July 2021. Ethical approval was obtained from the Ethics Committee of the Faculty of Medical Laboratory Sciences, Alneelain University. A total of 99 Sudanese children with confirmed retinoblastoma were included in the study. Inclusion criteria consisted of Sudanese nationality, age between 6 months and 6 years, a confirmed diagnosis of retinoblastoma based on clinical, radiological, and histopathological evidence, and the availability of sufficient FFPE tissue samples. Exclusion criteria included incomplete clinical data or poor-quality tissue unsuitable for molecular analysis. Diagnosis was based on clinical signs, transocular ultrasonography, fundus examination using the Reteam machine, and orbital CT/MRI imaging to determine tumor size, intraocular occupancy, and calcification. Final confirmation was performed by histopathological examination and immunohistochemical (IHC) analysis. Clinicopathological and demographic data—including patient age, gender, address, tumor stage, optic nerve involvement, tumor grade, and RB1 protein expression—were retrieved from medical records and the histopathology Laboratory Information System (LIS). Informed consent was obtained from the patients' guardians before enrollment.

2.2. DNA Extraction

Formalin-Fixed Paraffin-Embedded (FFPE) tissues of histologically confirmed retinoblastoma cases were retrieved from the pathology archive. Two to three sections, each with a thickness of 10 µm, were cut from each block using a rotary microtome. Excess paraffin was trimmed manually using a sterile scalpel, and the sections were placed into 2 mL Eppendorf tubes. Genomic DNA was extracted using the FlexiGene DNA Kit (Qiagen, Germany) according to the manufacturer's instructions. The extracted DNA was stored at –20 °C for downstream molecular analysis. DNA purity and concentration were evaluated using a NanoDrop 2000 ultra-micro spectrophotometer (Thermo Fisher Scientific, USA), and integrity was assessed using 2% agarose gel electrophoresis. Gels were visualized under a UV transilluminator equipped with a digital imaging system.

2.3. Primer Sequences

PCR primers were designed to amplify specific regions of the RB1 gene using NCBI Primer-BLAST, based on the gene sequence retrieved from the National Center for Biotechnology Information (NCBI) database. The amplification efficiency of each primer pair was optimized before use. The primer sequences and their respective melting temperatures and expected product sizes are summarized in Table **1**. These included inner primers specific for T, C, G, and A alleles and outer primers for general amplification. Product sizes ranged between 198 and 287 base pairs.

2.4. PCR Protocol for Detection of RB1 Exon 18 Gene Variations

To investigate exon 18 of the RB1 gene, three single nucleotide polymorphisms (SNPs)—rs137853292, rs772068738, and rs375645171—were

Table 1: Primer Sequences Used for Allele-Specific PCR of RB1 Gene and Expected Product Sizes

Primer Type	Primer Sequence (5′–3′)	Product Size (bp)	Melting Temperature (°C)
Forward Inner (T allele)	TTTCATCATGTTTCATATAGGAGTT	198	55
Reverse Inner (C allele)	GATTGTTTAATAAGATCAAATAAAGTTG	264	55
Forward Outer	TATAAGCGTTGAAGGTTATACATTT	198	55
Reverse Outer	CTTATGCTTAAAATCTATTTGCAGT	264	55
Forward Inner (G allele)	ACAATCAAAGGACCGAGCAG	260	60
Reverse Inner (A allele)	TCAAGGTGATCAGTTGGGCT	234	60
Forward Inner (T allele)	AATCTGCTTGTCCTCTTAATCTGCT	259	61
Reverse Inner (C allele)	GCTGCAGTGTGATTATTCTGGATAG	287	61

selected from the dbSNP database for allele-specific PCR. Each PCR reaction was prepared in a 20 µL volume, comprising 14 µL of distilled water, 1 µL of dimethyl sulfoxide (DMSO), 3 µL of template DNA, and 1 µL each of forward and reverse primers. PCR cycling was carried out for 35 cycles, with an annealing temperature set at 61°C. The resulting amplification products were separated using 2% agarose gel electrophoresis, and bands were visualized using an automated gel photo documentation system. After electrophoresis, PCR products were purified to remove unincorporated primers and nucleotides in preparation for sequencing.

2.5. DNA Sequencing

Purified PCR products were subjected to Sanger sequencing to identify RB1 gene variants. The products were first eluted in 30 µL of elution buffer and diluted at a 1:10 ratio with nuclease-free water. Sequencing reactions were prepared using the BigDye Terminator v1.1 kit (Applied Biosystems) and performed in both forward and reverse directions using the original PCR primers. The sequencing reactions were purified using the BigDye XTerminator Purification Kit (Life Technologies) and loaded onto a 3500 Genetic Analyzer (Life Technologies) for capillary electrophoresis. Resulting sequencing chromatograms were analyzed using Sequencing Analysis v5.4 and SeqScape v2.7 software (Life Technologies), and the obtained sequences were aligned with the RB1 reference sequence to detect any point mutations or polymorphic variants.

3. RESULT

3.1. Clinicopathological Features of Retinoblastoma Cases

Among the total of 99 cases of retinoblastoma included in the present study, 53.5% were female, while 46.5% were male. The majority of cases (79.8%) were suffering from unilateral lesions, while 20.2% were suffering from bilateral lesions. (55.6%) exhibit no nerve invasion, while (44.4%) show evidence of nerve invasion. The majority (58.6%) of cases were classified as T4, indicating advanced tumor stages. Additionally, there were cases classified as T2 (15.2%) and T3 (22.2%), representing intermediate stages, while only a small proportion of cases were classified as T1 (4.0%), indicating early-stage tumors. Among the total 99 cases recruited, the majority (69.7%) were classified as G3, indicating high-grade tumors. Additionally, a significant proportion (28.3%) were classified as G2, representing moderately differentiated tumors. A smaller percentage (2.0%) fell into the G1 category, denoting well-differentiated tumors. The majority of cases were from the Damazin state (15.2%), followed by Nyala (13.1%) and Al Obaid (12.1%). Other notable locations include Genina (12.1%) and Babanosa (11.1%). Together, these locations account for the majority of cases, with the remaining distributed across various other geographical locations. Table **2** summarizes all the clinicopathological and demographic variables.

3.2. DNA Sequencing

The DNA sequencing analysis revealed nucleotide changes in the selected PCR products. Sequencing data were analyzed using Sequencing Analysis v5.4 and SeqScape v2.7 software. The results showed clear differences when compared to the known RB1 reference sequence, indicating the presence of polymorphisms or mutations (Figure **1**).

Sanger sequencing identified multiple single nucleotide polymorphisms (SNPs) in exon 18 and the intronic region of the RB1 gene among Sudanese retinoblastoma patients. The identified SNPs include rs137853292 (C>G/T), rs375645171 (G>A),

Table 2: Demographic and Clinicopathological Features of Retinoblastoma Patients

Demographic and Clinicopathological Features	Frequency N= 99	Percentage %
Age/Month		
1- 12 Months	19	19.2
13 - 24 Months	30	30.3
25 - 36 Months	26	26.3
37 - 48 Months	18	18.2
49 - 60 Months	5	5.1
72 Months	1	1.0
Gender		
Female	53	53.5
Male	46	46.5
Geographical distribution (patients address)		
Al Obaid	12	12.1
Albagir	3	3.0
Al-Managel	5	5.1
Babanosa	11	11.1
Damazin	15	15.2
Fashir	9	9.1
Genina	12	12.1
Nyala	21	17.1
Omdurman	8	8.1
Port-Sudan	2	2.0
Taloody	5	5.1
Laterality		
Bilateral	20	20.2
Unilateral	79	79.8
Nerve Invasion		
No	55	55.6
Yes	44	44.4
Total	99	100.0
RB1 protein expression by IHC		
Negative	15	15.2
Positive	84	84.8
Tumour TNM Stage		
T1	4	4.0
T2	15	15.2
T3	22	22.2
T4	58	58.6
Tumour Grade		
G1	2	2.0
G2	28	28.3
G3	69	69.7

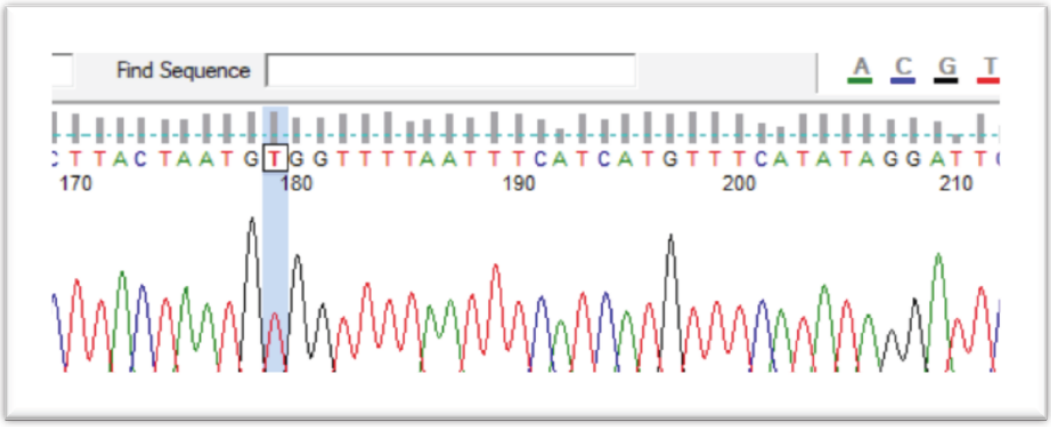

Figure 1: Chromatogram showing nucleotide sequence variation in the RB1 gene, as identified by Sanger sequencing. Peaks represent individual nucleotide bases, with the highlighted region indicating a detected mutation site compared to the reference sequence.

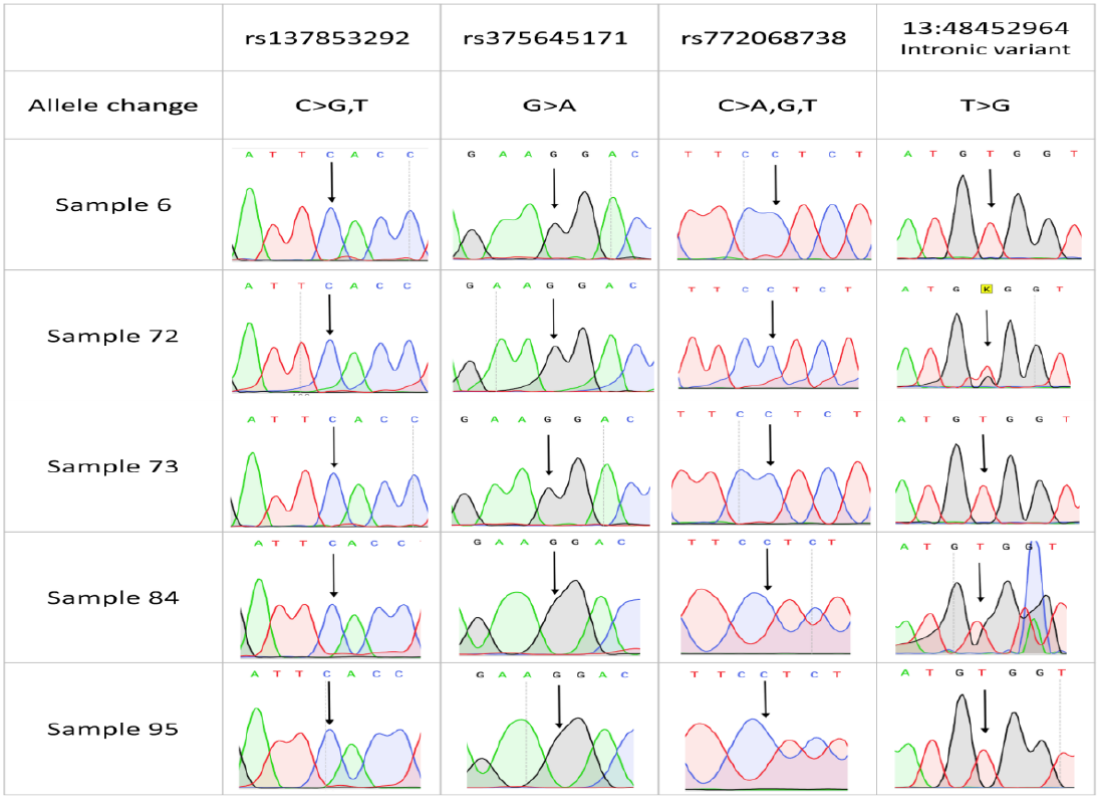

Figure 2: Sequencing analysis of the Hotpointed SNPs (rs137853292, rs375645171 and rs772068738) in Sudanese retinoblastoma patients.

rs772068738 (C>A/G/T), and an intronic variant at position 13:48452964 (T>G). as show in Figure (**2**) provides a comparative view of the chromatograms from five patient samples (Sample 6, 72, 73, 84, and 95), each demonstrating representative nucleotide substitutions at these loci. In the first column (rs137853292), clear C-to-G or C-to-T substitutions are seen, indicated by overlapping peaks and arrows. In the second column (rs375645171), a G-to-A substitution is consistently visible across all five samples. The third column (rs772068738) shows multiallelic variation.

3.4. RB Protein Expression Using IHC Analysis

Immunohistochemical analysis of RB1 protein expression revealed that 84 out of 99 cases (84.8%) showed positive staining for the monoclonal antibody specific to RB1 protein, while 15 cases (15.2%) were negative (Table **3**). Among the positive cases, scoring of IHC slides demonstrated that 4% of tumors exhibited

Table 3: RB1 Protein Expression Status in Retinoblastoma Cases (Positive vs. Negative)

RB1 Protein Expression (IHC)	Frequency	Percentage (%)
Negative	15	15.2
Positive	84	84.8
Total	99	100

Table 4: Expression Intensity of RB1 Protein in Positive Retinoblastoma Cases

Expression Level	Frequency	Percentage (%)
Weak Expression	4	4
Moderate Expression	21	25.3
Strong Expression	59	70
Total	84	100

weak expression, 25.3% showed moderate expression, and 70% displayed strong expression (Table **4**). Notably, higher RB1 expression was observed in tumors with extensive necrosis.

4. DISCUSSION

In the present study, we investigated the clinicopathological features and RB1 gene polymorphisms in 99 Sudanese patients with retinoblastoma. Our findings provide valuable insights into the clinical presentation and molecular characteristics of this disease within a developing country context.

The age of onset in our cohort ranged from 1 to 72 months, which aligns with a previous study reporting an onset between 1 and 60 months [10]. We observed a slightly higher prevalence among females (53.5%) compared to males (46.5%), whereas earlier reports demonstrated nearly equal gender distribution in a cohort of 50 retinoblastoma patients [10].

A notable finding was the predominance of unilateral cases (79.8%) over bilateral cases (20.2%), which is consistent with the literature, as unilateral retinoblastoma is more common and typically sporadic in nature [11,12]. Bilateral retinoblastoma, on the other hand, is often associated with inherited or de novo germline mutations in the RB1 gene. Some studies have reported a relationship between advanced paternal age and the development of bilateral retinoblastoma, suggesting an increased likelihood of de novo germline mutations with older parental age, though findings remain controversial [11-13]. Given the high rate of unilateral involvement in our study, we suggest that the majority of cases were likely sporadic.

Optic nerve invasion, an important marker of disease progression, was observed in 44.4% of cases. Postlaminar optic nerve invasion is strongly associated with poor prognosis, including recurrence and systemic metastasis [14-16]. This suggests a high likelihood of adverse clinical outcomes in nearly half of the cohort. Previous studies have identified clinical predictors of optic nerve invasion, such as elevated intraocular pressure, vitreous hemorrhage, exophytic growth pattern, and tumor thickness ≥15 mm [17].

A significant proportion of patients (58.6%) presented with T4 tumors according to the TNM classification, reflecting advanced disease stages. This is of clinical concern, as advanced tumor staging is associated with high-risk histopathologic features and may warrant consideration for adjuvant chemotherapy to improve survival [18].

Geographically, the distribution of cases varied, with the majority originating from Damazin, Nyala, and Al-Obaid. These disparities may reflect differences in access to healthcare, awareness, environmental exposures, or regional genetic predispositions. A study conducted in China identified parental living conditions, chemical exposure (especially paternal), and maternal contact with pets before pregnancy as independent risk factors for retinoblastoma development [19]. While our study did not investigate environmental or socioeconomic risk factors directly, the observed geographic clustering may indicate an area for future epidemiological studies.

RB1 protein expression analysis by IHC showed positive expression in 84.8% of cases, while 15.2% were negative. These findings are consistent with other

cancers such as oral squamous cell carcinoma, where RB1 expression was found to increase progressively from normal tissue through precancerous lesions to malignancy [20]. Among RB1-positive cases in our cohort, 70% showed strong expression, 25.3% had moderate expression, and 4% displayed weak expression. However, statistical analysis did not reveal a significant association between RB1 expression and tumor grade, tumor stage, or optic nerve invasion.

4.1. Limitations

This study has several limitations. First, no data were collected on family history, which could have helped differentiate hereditary from sporadic cases. Second, although sequencing was performed, there was no functional analysis of the identified RB1 polymorphisms to determine their biological impact. Third, the geographic scope was limited to two centers in Khartoum State, and the findings may not be generalizable to all regions of Sudan. Additionally, the sample size, although substantial, limits subgroup analysis for rare clinical or genetic features.

4.2. Clinical Implications

Despite these limitations, our study has important clinical implications. The identification of recurrent RB1 polymorphisms in Sudanese children with retinoblastoma highlights the potential for RB1 genetic screening as a tool for early diagnosis and risk assessment. In regions with limited resources, integrating genetic screening with routine clinical and imaging evaluations could aid in stratifying patients for timely intervention, especially in families with suspected hereditary cases. Furthermore, raising awareness about high-risk clinical features—such as advanced tumor staging and optic nerve invasion—can support the development of standardized treatment protocols and referral pathways, ultimately improve prognosis and reducing treatment delays.

5. CONCLUSIONS

Overall, our comprehensive characterization of clinic-pathological features, RB1 protein expression, and genetic alterations provides valuable insights into the complexity of retinoblastoma and underscores the importance of improving diagnosis, prognosis, and treatment strategies.

RECOMMENDATIONS

Based on our findings, we recommend the introduction of routine RB1 genetic screening for pediatric eye tumor cases in Sudan, particularly for early diagnosis and risk stratification. Future studies should be expanded to include larger, multicenter cohorts with broader geographic representation. Additionally, incorporating family history data, functional assays of identified polymorphisms, and longitudinal follow-up would provide deeper insights into disease progression, treatment response, and genetic inheritance patterns. Establishing standardized national protocols for RB1 testing and histopathological evaluation could significantly improve early detection and clinical outcomes.

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CONFLICTS OF INTEREST

No Conflicts of interest.

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REFERENCES

[1] Ibrahim NO, Saleem M, Eltayeb E, Mekki S, Elnaim EG, Idris AB, Bashir S, Salim MA, Hassan MA. Molecular genetic analysis of RB1 gene among Sudanese children with retinoblastoma. bioRxiv 2019. https://doi.org/10.1101/519306

[2] Abdalla MA, Mohammed Z. The pattern of pediatric malignancy and outcome in Khartoum Oncology Hospital, Sudan: a 15-year single-center experience. Pediatr Hematol Oncol J 2024; 9(1): 1-8. https://doi.org/10.1016/j.phoj.2023.12.006

[3] Kato MV, Ishizaki K, Shimizu T, Ejima Y, Tanooka H, Takayama J, Kaneko A, Toguchida J, Sasaki MS. Parental origin of germ-line and somatic mutations in the retinoblastoma gene. Hum Genet 1994; 94(1): 31-8. https://doi.org/10.1007/BF02272838

[4] Kadam-Pai P, Su XY, Miranda JJ, Soemantri A, Saha N, Heng CK, Lai PS. Ethnic variations of a retinoblastoma susceptibility gene (RB1) polymorphism in eight Asian populations. J Genet 2003; 82(1-2): 33-7. https://doi.org/10.1007/BF02715879

[5] Viatour P, Sage J. Newly identified aspects of tumor suppression by RB. Dis Model Mech 2011; 4(5): 581-5. https://doi.org/10.1242/dmm.008060

[6] Burkhart DL, Sage J. Cellular mechanisms of tumour suppression by the retinoblastoma gene. Nat Rev Cancer 2008; 8(9): 671-82. https://doi.org/10.1038/nrc2399

[7] Cavenee WK, Leach R, Mohandas T, Pearson P, White R. Isolation and regional localization of DNA segments revealing polymorphic loci from human chromosome 13. Am J Hum Genet 1984; 36(1): 10-24.

[8] Anaya-Pava EJ, Nares-Cisneros J, Cárdenas-Hernández RI, Jaramillo-Rodríguez Y, Zambrano-Galván G. Study of polymorphisms in the TP53 and RB1 genes in children with retinoblastoma in northern Mexico. Mol Vis 2017; 23: 20-25.

[9] Zhang Y, Wang Y, Huang D, Ma J, Zhang W, Gu H, Zhou Y, Yi Y, Zhang P. Correlation between family RB1 gene pathogenic variant with clinical features and prognosis of retinoblastoma under 5 years old. Dis Markers 2021; 2021: 9981028. https://doi.org/10.1155/2021/9981028

[10] Azimi F, Naseripour M, Sedaghat A, Kachoei ZA, Khakpoor G. Association of RB1 rs9568036 and CDKN1A rs1801270 polymorphisms with retinoblastoma susceptibility. J Curr Ophthalmol 2022; 34(3): 328-332. https://doi.org/10.4103/joco.joco_125_22

[11] Mills MB, Hudgins L, Balise RR, Abramson DH, Kleinerman RA. Mutation risk associated with paternal and maternal age in a cohort of retinoblastoma survivors. Hum Genet 2012; 131(7): 1115-22. https://doi.org/10.1007/s00439-011-1126-2

[12] Saremi L, Imani S, Rostaminia M, Nadeali Z. Parental age-related risk of retinoblastoma in Iranian children. Asian Pac J Cancer Prev 2014; 15(6): 2847-50. https://doi.org/10.7314/APJCP.2014.15.6.2847

[13] Lloyd P, Westcott M, Kaliki S, Ji X, Zou Y, Rashid R, *et al.* The role of maternal age & birth order on the development of unilateral and bilateral retinoblastoma: a multicentre study. Eye (Lond) 2023; 37(5): 966-70. https://doi.org/10.1038/s41433-022-01992-w

[14] Chantada GL, Doz F, Orjuela M, Qaddoumi I, Sitorus RS, Kepak T, *et al.* World disparities in risk definition and management of retinoblastoma: a report from the International Retinoblastoma Staging Working Group. Pediatr Blood Cancer 2008; 50(3): 692-4. https://doi.org/10.1002/pbc.21427

[15] Gupta R, Vemuganti GK, Reddy VA, Honavar SG. Histopathologic risk factors in retinoblastoma in India. Arch Pathol Lab Med 2009; 133(8): 1210-4. https://doi.org/10.5858/133.8.1210

[16] Sastre X, Chantada GL, Doz F, Wilson MW, de Davila MT, Rodríguez-Galindo C, *et al.* Proceedings of the consensus meetings from the International Retinoblastoma Staging Working Group on the pathology guidelines for the examination of enucleated eyes and evaluation of prognostic risk factors in retinoblastoma. Arch Pathol Lab Med 2009; 133(8): 1199-202. https://doi.org/10.5858/133.8.1199

[17] Shields CL, Shields JA, Baez K, Cater JR, de Potter P. Optic nerve invasion of retinoblastoma: metastatic potential and clinical risk factors. Cancer 1994; 73(3): 692-8. https://doi.org/10.1002/1097-0142(19940201)73:3<692::AID-CNCR2820730331>3.0.CO;2-8

[18] Yousef YA, Al-Hussaini M, Mehyar M, Sultan I, Jaradat I, AlRawashdeh K, et al. Predictive value of TNM classification, International Classification, and Reese-Ellsworth staging of retinoblastoma for the likelihood of high-risk pathologic features. Retina 2015; 35(9): 1883-9. https://doi.org/10.1097/IAE.0000000000000547

[19] Yang YQ, Li J, Yuan HF. Epidemiology and risk factors of retinoblastoma in Chongqing area. Int J Ophthalmol 2016; 9(7): 984-8.

[20] Thomas S, Balan A, Balaram P. The expression of retinoblastoma tumor suppressor protein in oral cancers and precancers: a clinicopathological study. Dent Res J (Isfahan) 2015; 12(4): 307-14. https://doi.org/10.4103/1735-3327.161427

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