Zahedan J Res Med Sci. 2022 October; 24(4):e117490. https://doi.org/10.5812/zjrms-117490.

Published online 2022 October 3. **Research Article**

The Concordance of Paternal and Maternal Retinoblastoma-1 Gene Mutation Pattern with Clinical Manifestation and Disease Staging in Patients Suffering from Retinoblastoma

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Received 2021 July 13; **Revised** 2021 August 14; **Accepted** 2021 September 08.

Abstract

Background: Retinoblastoma (RB) is a common neoplastic disease in children, leading to high mortality if not diagnosed and treated timely. Mutations in both versions of the Retinoblastoma1 (RB1) gene are responsible for this disease. A wide range of mutations has been reported throughout the RB1 gene.
Objectives: The present study aimed to assess the concordance of paternal and maternal RB-1 gene mutation with clinical manifestation and disease staging in patients suffering from RB.
Methods: This cross-sectional study was performed on 23 patients with unilateral or bilateral RB. Paternal and maternal peripheral blood samples were extracted for genome analysis. Information related to clinical manifestations and disease staging was collected from the patients' hospital records. Multiplex-ligation dependent probe amplification (MLPA) method or Sanger sequencing method was employed to assess the gene mutation and its genomic pattern.
Results: No significant association was revealed between the presence of both maternal and paternal RB1 gene mutations and the disease staging, while the study could show a significant relationship between the presence and heterozygous pattern of RB1 gene mutation and the presence of disease-related clinical manifestations that bilateral involvement was strongly associated with the presence of a heterozygous pattern of gene mutation compared to unilateral involvement (P = 0.001).
Conclusions: This study showed a significant correlation between the presence of RB1 gene mutation and bilateral involvement in RB, but the association between the disease staging and gene mutation remains insignificant.

Keywords: Mutation, Retinoblastoma-1 Gene, Phenotype, Retinoblastoma, Phenotype; Retinoblastoma; Iran

1. Background

Retinoblastoma (RB) is the most common intraocular malignancy among children (1) that is mainly diagnosed during a routine eye examination with a white pupil reflex, known as leukocoria (2). This disease occurs in both hereditary and non-hereditary forms (3) It seems that the prevalence of non-hereditary (sporadic) type in developing countries, especially poor countries, is higher than in other countries (4). The disease most often occurs in children, usually before the age of two (5). The disease has two common phenotypes, including (A) non-hereditary phenotype that is unilateral (in one eye) and focal and occurs in 2/3 of patients. This means that mutations in the RB1 gene are present only in ocular cells and cannot be transmitted to the next generation (6) and (B) hereditary phenotype (autosomal dominant) that is bilateral (in both eyes) and multifocal and occurs in 1/3 of patients. This means that mutations in the RB1 gene are present in all cells of the body, including reproductive cells (sperm or ovule) (7) and rarely occur in triplet (a combination of unilateral or bilateral RB) (1). In the non-hereditary form, the risk of disease transmission is only 5%, while in the hereditary form, the risk increases to 50% (8). The only cause of RB is a mutation in the RB1 gene following point mutations (non-sense (frameshift, splicing missense) or mutation in the promoter region

of the gene), and to date, no other factors have been reported to affect the onset of the disease (9, 10). There are four possible sources for RB disease: retinal cells, retinal stem cells (progenitor), neuronal, and glial cells, and finally, mitotic cells are involved in the production of retinal cells in humans (11). According to the international classification system, which is a classification system for intraocular tumors based on location, multifocal, and tumor size, intraocular RB is divided into five groups of A to E, including A group (Very low-risk), B group (Low-risk), C group (Moderate-risk), D group (High-risk), and E group (Very high-risk eyes) (12, 13). The disease follows a mutation in the RB1 gene, and about 40% of patients inherit the first mutation as a germline. In most of these people, the second mutation occurred somatically in a number of retinal cells, which mainly causes bilateral involvement of the disease (13). The disease can be transmitted to the next generation as an autosomal dominant trait with high penetration, but in the remaining 60% of patients, both mutations responsible for the disease occur somatically in retinal cells, which lead to the formation of unilateral involvement of the disease (13).

2. Methods

This study was conducted as cross-sectional analytical research. Thirty patients with a history of RB were consecutively entered into the study. Inclusion criteria were ocular RB disease and no systemic and secondary neoplastic diseases. The population consisted of children aged one month to 17 years referred to Rasoul Akram Research Center between 2018 and 2019 after being diagnosed with RB. Before starting the study, a checklist was designed to collect the baseline information (including age at diagnosis, body weight, family history of the disease, clinical manifestations specific to RB, and treatment protocols) by reviewing the patients' recorded files. Also, the grade of RB based on its prognosis was classified according to the international intraocular RB classification previously described in detail. Briefly, A group was classified as very low-risk, B group as low-risk, C group as moderate-risk, D group as high-risk, and E group as very high-risk (13).

For genomic assessment, about five cc of venous blood sample was taken from the patients and transferred to a centrifuge tube previously filled with 300 microliters of EDTA solution. The DNA of the patients was extracted from their white blood cells by salting out technique. Then gene deletions or duplications were evaluated using Multiplex-ligation dependent probe amplification (MLPA) method. If a gene mutation was not detected, the whole DNA samples extracted in the gene region were subjected to gene sequencing using Sanger method, and the type of mutation was determined (Table 1). Finally, the relationship of the presence/absence of mutation in RB1 gene, type of mutation, and gene deletion and phenotypic parameter such as clinical characteristics of patients (grade of ocular involvement) was assessed. For statistical analysis, results were presented as frequency (percentage) for categorical variables. Categorical variables were compared using chi-square test. For the statistical analysis, the statistical software SPSS version 23.0 for windows (IBM, Armonk, New York) was used.

Table 1. Different Types of Mutations Detected in RB1 Gene

Chromosome Mutation	No. (%)
None	13.0 (3)
Deletion 15 - 16	4.3 (1)
Deletion 2 - 26	4.3 (1)
E = 1 A/T AAA>TAA Lys/stop	4.3 (1)
E = 3 c.380+1 G>T Splice site	4.3 (1)
E = 20 c.del -C2020 p.Pro 674 Gln Fsx2*	4.3 (1)
E = 4 c.453 ins T	4.3 (1)
E = 17 c. 1654 C>T p/Arg 552 X*	4.3 (1)
E = 9 307 Thr> Ile ACA>ATA 920C>T	4.3 (1)
E = 11 R358X	4.3 (1)
E = 6 c.607+1 G>A	4.3 (1)
E = 7 c.610 G>T p.E204*	4.3 (1)
E = 14 CGA>TGA Arg 455 stop c.1363 C>T	4.3 (1)
E = 4 c.386 A>C CAT>CCT His 129>PRO Fs*1	4.3 (1)
E = 9 307 Thr>Ile ACA>ATA 920 C>T	4.3 (1)
E = 23 c.2349 delT P.Pro 783 Pro fx26*	4.3 (1)
E = 18 c.ins ATCTGCT C C.1761-1767 GAA>GAT Glu587 AspFX21	4.3 (1)
E = 19 c.1954 A>T p.Lys 652X AAA>TAA	4.3 (1)
E = 19 c.887-888 delGA GAG>GAC Glu629AspfsX22	4.3 (1)
E = 19 c.1960+1 splice Del G	4.3 (1)
E = 1 c.C80ins G CCC>CGC Pro27>R	4.3 (1)
Total	100.0 (23)

3. Results

In total, 69.6% of the samples in this study aged less than one year, 17.4% aged between one to two years, and 13% aged more than two years. Also, 56.5% were male

and 43.5% were female. The details of the classification of disease in left and right eyes are summarized in Table 2. In this regard, the right eyes were classified into the E group and left eyes into the group D of staging classification. As shown in Table 3, no significant association was revealed between the presence of both maternal and paternal RB1 gene mutations and the disease staging in both right and left eyes. In total, investigating fathers' and mother's gene-related polymorphisms, 21.7%, and 8.7% had heterozygous patterns, and wild type was only observed in 4.3% of mothers. The study showed a significant relationship between the presence and type of RB1 gene mutation. The presence of disease-related manifestations that bilateral involvement was strongly associated with the presence of heterozygous pattern of RB1 gene mutation compared to unilateral involvement (P = 0.001) (Table 4).

Table 2. The Disease Staging in Right and Left Eyes According to Paternal and Maternal RB1 Gene Mutation Pattern

Staging	Right Eye	Left Eye
Class A	2 (8.7)	3 (13.0)
Class B	4 (17.4)	2 (8.7)
Class C	4 (17.4)	2 (8.7)
Class D	4 (17.4)	11 (47.8)
Class E	9 (39.1)	5 (21.7)

4. Discussion

Molecular examination of RB1 gene mutations can greatly improve the process of disease control and management; thus, other high-risk individuals in the patient's family do not need to undergo painful and costly tests performed under anesthesia. Furthermore, only the necessary studies will be performed on individuals who carry mutations in the RB1 gene (14). According to the results, it can be said that RB is higher in males than females, and the most common age group was less than one year group. Nabie et al. showed that the frequency of unilateral RB1 presentation (57.7%) was slightly higher than bilateral presentation (42.5%) in children with RB1 in north-west Iran; however, the results of this study showed that bilateral presentation (82.60%) was significant compared to unilateral presentation of RB1 (17.39%) (15). Shahraki et al. performed a comprehensive genetic screening on 106 patients who were diagnosed with RB in Iran. According to their results, the mean age at the time of diagnosis was 12.7±10.5 for RB with a positive family history. They also demonstrated 38 different causative RB1 mutations among patients, with an RB1 mutation detection rate of 65.8%. In this study, we detected more than 19 different mutations among RB1 patients (16). Ghassemi and colleagues showed that the most common presenting stage of RB1-carrying patients at IAC therapy was the D group, reaching up to 75%, followed by the E group and C group at the first visit. Interestingly, clinical examination of left eye in our patients demonstrated that D group was the most common diagnosed class (47.8%) followed by E group (21.7%) (17). There was no significant relationship between genomic mutations in parents, mutation, and phenotype of patients, and also between the type of genetic mutation and disease manifestations. This means that even if genetic aberration causes all RB tumors, it means that neither all patients have inherited the disease nor the next generation can inherit all cases. Approximately 10% of people with hereditary RB inherit the RB1 mutation from their parents. This means that most people with hereditary RB are the first person in their family who have RB1 mutation.

For example, some RB tumors are caused by the RB1 mutation (13). Most patients with inherited RB develop retinal tumors, either benign (retinoma), or malignant (RB), both of which are caused by the loss of the second RB1 allele in the sensitive retinal cell. However, RB may be inherited, but no retinal tumors develop (18). The disease follows a mutation in the RB1 gene, and about 40% of patients inherit the first mutation as a germline. In most of these people, the second mutation occurred somatically in a number of retinal cells, which mainly causes bilateral involvement of the disease (13). The disease can be transmitted to the next generation as an autosomal dominant trait with high penetration, but in the remaining 60% of patients, both mutations responsible for the disease occur somatically in retinal cells, which lead to the formation of unilateral involvement of the disease (13).

4.1. Conclusions

This study showed a statistically significant correlation between the presence of RB1 gene mutation and clinical specific manifestations of RB, but the association between the disease staging and gene mutation remains insignificant.

Footnotes

Authors' Contribution: Study concept and design: SH.SHM., and M.F.; Analysis and interpretation of data:

Table 3. The Association Between Disease Staging and Paternal and Maternal RB1 Gene Mutation Pattern

Genetic Pattern	Paternal					Maternal				
	A	B	C	D	E	A	B	C	D	E
Right eye										
No	1	2	3	2	7	1	2	4	4	7
N/A	1	1	0	0	1	1	0	0	0	1
Heterozygous	0	1	1	2	1	0	1	0	0	1
Wild type	-	-	-	-	-	0	1	0	0	0
P-value			0.36					0.45		
Left eye										
No	1	1	2	8	3	2	1	2	10	3
N/A	1	1	0	0	1	0	1	0	0	1
Heterozygous	1	0	0	3	1	1	0	0	1	0
Wild type	-	-	-	-	-	0	0	0	0	1
P-value			0.57					0.51		

Table 4. The Association Between Disease Clinical Pattern and RB1 Gene Mutation Pattern [a]

Clinical Involvement	Homozygous Pattern	Heterozygous Pattern
Right-sided	0	2
Left-sided	2	0
Bilateral	0	19

[a] $P < 0.001$

SH.SHM., and M.JM. and MJ.A.; Drafting of manuscript: SH.SHM., M.JM., MJ.A.; Critical revision of the manuscript for intellectual content: M.F., A.S., SH.SHM.; Statistical analysis: SH.SHM., M.JM., MJ.A.

Conflict of Interests: The authors declare that they have no conflict of interest.

Funding/Support: This study was supported in part by a charity association (MAHTA laboratory).

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