

## RESEARCH ARTICLE

# Parental Age-Related Risk of Retinoblastoma in Iranian Children

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### Abstract

**Background:** Retinoblastoma is a rare malignant intraocular neoplasm. About 90% of cases feature a germline mutation in the *RBI* gene and these will develop retinoblastoma during their early childhood. An association between mutations in germline cells and aging has been demonstrated. This suggests a higher incidence of childhood cancer including retinoblastoma among children of older parents. **Materials and Methods:** In the present study we aimed to determine the association of paternal and maternal age with an increased risk of retinoblastoma in a case-control study in Iranian population. The study was carried out on 240 persons who were born during 1984-2012 in Mahak and Mofid hospitals in Tehran, Iran. The statistical analysis included studying the mean age of parents and in order to know whether parental age of patients is different from parental age of control group, (t-test) compare averages test is used perfectly. By binary logistic regression, odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. **Results:** The results of statistical analysis including the study of mean parental age by the use of (t-test) compare averages test showed a significant difference between parental ages of patients and controls. Logistic regression showed that coefficients were significant for maternal but not paternal age. **Conclusions:** Our findings indicate that advanced maternal age can increase the risk of retinoblastoma in offspring, but the paternal age has no significant effect.

**Keywords:** Retinoblastoma - parental age - increased risk - statistical analysis

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### Introduction

Retinoblastoma is a rare malignant intraocular neoplasm that originates in the primordial retinal cells. Most often it occurs in children, usually before the age of two years (Khetan et al., 2011; Abramson et al., 2012). Two types of retinoblastomas have been defined: those are due to genetic mutations and the sporadic retinoblastomas. The hereditary form is divided into two groups, those that arise in children who have a parent with retinoblastoma or a family history of disease, indicating that one of the parents must be a carrier of the *RBI* gene (Dimaras, 2012 and Howlader, 2013). (Familial retinoblastoma) and those in which no other family members are affected, the disease occurs as the result of a new mutation and the patient is the first person in the family with retinoblastoma. (Sporadic heritable retinoblastoma) (MacCarthy et al., 2012).

The disease may occur unilaterally (in one eye), bilaterally (in both eyes) and rarely trilateral (combination of unilateral or bilateral retinoblastoma) (Friend et al., 1986; Shah et al., 2013). Both familial and sporadic heritable types are more likely to have bilateral status and occur in the first year of life, while the sporadic retinoblastomas are more likely to be unilateral and occur after the first year of life. It has been estimated that in 60% of affected children, retinoblastoma is unilateral and 15%

of these cases are hereditary and in about 40% of cases, the disease is bilateral.

The majority of heritable cases have sporadic retinoblastoma and there is no family history (MacCarthy et al., 2009). Retinoblastoma is caused by the mutations in both alleles of the retinoblastoma gene, *RBI* at 13q14. These mutations occur prezygotically in germline (initial mutation in bilateral disease) or postzygotically (second hit) occur due to the somatic *RBI* mutation during fetal development or early infancy (Broaddus, 2009). The disease occurs 1 in 15,000 to 20,000 live births (Marees, 2008). In about 90% of cases carrying a germline mutation in *RBI* gene will develop retinoblastoma during their early childhood (Yu et al., 2009; MacCarthy et al., 2011). The familial retinoblastoma has an autosomal dominant inheritance (Mehta et al., 2012). The number of new mutations in germline cells increases with age (Daniel, 2013). Both advanced maternal age and advanced paternal age have been associated with a number of congenital syndromes, including a number of cancers like retinoblastoma (Crow, 2000). The aim of the present study was to determine the association of paternal and maternal age with an increased risk of retinoblastoma in offspring in a case-control study. To date this is the first study has been conducted in Iranian population.

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## Materials and Methods

The study was carried out on children who were born during 1984-2012 in Iran. These persons belonged to various ethnicities from different provinces of Iran. For sampling process, one of the specialized cancer hospitals named Mahak was selected and also among retinoblastoma patients, 120 cases were selected randomly. For control group, Mofid hospital was selected and 120 healthy individuals as control subjects were selected randomly. We extracted the date of birth of these 240 children born during the period from 1984 to 2012 and their parental age at birth. After informed consent had been obtained from all participants, the statistical analysis was performed.

The statistical method in this regard includes studying the mean age of parents and in order to know whether parental age of patients is different from parental age of control group, (t-test) compare averages test is used perfectly. Using regression, it was assessed whether parental age is effective in being patient or not? Since that the dependent variable is a qualitative variable, logistic regression is used and the parental ages are considered as regressors. By the binary logistic regression, odds ratios (ORs) and 95% confidence intervals (CIs) were calculated.

## Results

Data on parental ages at birth, during the period 1984-2012, were obtained for fathers and mothers of retinoblastoma patients and control subjects. The descriptive statistics of sample and control groups and the distributions of both paternal and maternal ages for all cases are shown in Table 1. A total of 240 subjects were studied among which 120 retinoblastoma patients that were pathologically diagnosed between 1984 to 2012 attending the Mahak hospital as well as 120 control subjects attending Mofid hospital both located in Tehran, Iran.

The results of statistical analysis including the study of parental average age by the use of (t-test) compare averages test showed a significant difference between parental age of patients and parental age of control group. T-test was performed for mean parental age of patient and control groups and the results are shown in Table 2. As it is obvious, mean maternal age in patient group is 3.45 years higher than mean maternal age of control group and the average paternal age of patient group is 1.83 years higher than average paternal age in control group. According to tests shown in Table 2 the difference of averages is statistically significant in level 5%. Although

**Table 1. Statistical Characteristics of Patient and Control Groups**

Control or patient	N	Mean age	Std. Deviation	Std. Error Mean	
Maternal age	P*	120	28.1083	6.51668	0.59489
	C**	120	24.65	4.82927	0.44085
Paternal age	P*	120	33.3583	5.93918	0.54217
	C**	120	31.525	5.03761	0.45987

\*Patients, \*\*Control

**Table 2. T-Test Averages Comparative Test for the Mean Parental age of Patient and Control Groups with the Assumption of Not Equal Variances**

	Equal variances not assumed	T-test for equality of means	
		Maternal age	Paternal age
	t	4.671	2.579
	Df	219.418	231.828
	p	0.000*	0.011*
Mean difference		3.45833	1.83333
Std error difference		0.74043	0.71093
95% CI of the difference	Lower	1.99906	0.43261
	Upper	4.91761	3.23405

**Table 3. Binary Logistic Regression Which in the First Step the Paternal and Maternal Age is Considered as Regressor and in the Second Step Only the Maternal Age is Considered as Regressor**

	Wald	df	p value	OR	95%CI for OR	
					Lower	Upper
Step 1a						
PaternalAge	0.803	1	0.37	0.97	0.906	1.037
MaternalAge	13.751	1	0.000*	1.132	1.06	1.209
Constant	7.797	1	0.005*	0.103		
Step 2a						
MaternalAge	18.922	1	0.000*	1.109	1.059	1.162
Constant	18.15	1	0.000*	0.065		

\*a: Variable(s) entered on step 1: Paternal Age, Maternal Age

the paternal age of patient group in our study sample is significantly higher than the paternal age of control group but this is due to the fact that women usually marry with men who are some years older than themselves, thus the significant difference among paternal age in fact comes from the difference between maternal age. To diagnose and emphasize this theorem; the two-step regression is used and the parental ages are considered as repressor's. Logistic regression results showed that some coefficients are significant and maternal age is significantly effective on catching disease but paternal age is not. Binary logistic regression results are observed in Table 3.

As it is observed in the first step the age coefficient of father is not significant statistically, so it is not useful in model and it is deleted; this result emphasizes our idea about paternal age effect on retinoblastoma, so the second step of regression is performed with just the age of mother as regressors; which the coefficients are significant in level 1% and odd ratio of maternal age is more than 1, this means that the changes of age of mothers can explain the development of retinoblastoma and it is not necessary to enter the ages of fathers to model.

Finally it can be concluded that advanced maternal age can increase the risk of retinoblastoma in offspring. But the paternal age has not significant effect on retinoblastoma risk.

## Discussion

An association between mutations in germline cells and aging has been demonstrated. This suggests a higher incidence of childhood cancer including retinoblastoma

among children of older parents (Yip, 2006). The independent associations of maternal and paternal age with increased risk of retinoblastoma are difficult to separate, due to their strong correlation.

A number of mechanisms have been suggested through which advanced maternal age could affect childhood cancer risk. Germline mutations occur less frequently in oocytes (maybe because of the fact that oocytes undergo far fewer cell divisions during gametogenesis) in comparison to sperm. Other mechanisms including age related differential gene expression in oocytes of older versus younger mothers due to promoter DNA methylation and de novo epimutations in oocyte genes that could be transmitted to offspring (Hitchins, 2007 and Esteller, 2008). Some other mechanisms could also be considered. Use of assisted reproductive technology, which increases with maternal age, could elevate the cancer risk in offspring conceived in this way (Lightfoot et al., 2005; Neelanjana et al., 2008; Johnson et al., 2009). In addition, age related changes in hormonal levels during pregnancy, that could increase the risk of cancer in the offspring can be considered (Dockerty et al., 2001).

In addition to maternal age factor, epidemiological observations suggest that parental age is related to the genesis of certain anomalies. Some studies conducted in this regard, concluded that there seems to be a paternal age effect in bilateral but not in unilateral cases, although the extent of the effect is much smaller than in the other anomalies (Katy, 2013). The most likely mechanism for advanced paternal age effect is de novo germline mutations. Mostly mutations have been shown to originate from the paternal germline (Dimaras et al., 2012). DNA investigations of some retinoblastoma patients suggest that new germline mutations principally have paternal origin. In the literature, the existence of an association of retinoblastoma with parental age is still controversial. Most of the available studies in this regard are based on a small number of cases (Johnson et al., 2009). Previous large studies, have detected associations of parents' age with retinoblastoma, but led to different results: Pellié et al. have established a paternal age effect, Der Kinderen et al. have found a maternal age effect, Matsunaga et al. have suggested only an age effect for fathers older than 35 years, Moll et al. established both paternal and maternal age effect and Johnson et al. mentioned maternal age effect (Zand, 2012; Katy, 2013).

The aim of the present study was to investigate the association of parental age, with the birth of retinoblastoma patient. Our findings indicate the association between advanced maternal age and increased risk of retinoblastoma for the period 1984-2012 in Iran.

This confirms our hypothesis that parental age is a risk factor for retinoblastoma. Suggesting that maternal age may be as a risk factor for retinoblastoma on a population level. Although the paternal age of patient group in our study sample is significantly higher than the paternal age of control group but this is due to the fact that women usually marry with men who are some years older than themselves, thus the significant difference among paternal age, in fact comes from the difference between maternal ages.

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