ORIGINAL ARTICLE

GENETIC PROFILE OF RETINOBLASTOMA PATIENTS FROM A REFERRAL HOSPITAL IN GUJARAT

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ABSTRACT

Background & objectives: Retinoblastoma is the most common intraocular malignancy of infant and childhood. It is the first disease for which a genetic etiology of cancer has been described and the first suppressor gene identified. The chromosomal abnormality found in retinoblastoma cases is deletion of 13q14. The aim of this study was to carry out karyotypic study in Retinoblastoma cases and identifying nature of chromosomal abnormalities in these patients to determine recurrence risks to assist genetic counseling.

Materials and Methods: Karyotypic study was perform by Trypsin- Giemsa Banding for 17 unilateral and 8 bilateral retinoblastoma patients (total 25) for that their blood samples were taken. A prior written consent was taken from the parents of these patients.

Results: Out of 25 cases of Retinoblastoma, one female (4%) had 13q14 deletion and 24 (96%) showed normal chromosomal constitution and not a single case of translocation and mosaicism were found.

Conclusion: Karyotyping is the simplest and affordable genetic test for most of the retinoblastoma families especially in a developing country like India. Genetic testing is crucial for accurate risk prediction for retinoblastoma in close relatives of probands and provides a basis for genetic counseling.

Key Words: Retinoblastoma, Chromosomal abnormalities, 13q14 deletion, Genetic counseling, Karyotype

INTRODUCTION

Retinoblastoma is the most common malignant intraocular tumour in the paediatric age group[1] with an incidence of 1:12000 to 1:23000 live births amongst different races. The tumour in retinoblastoma arises from the retinal precursor cells[8] and therefore manifests as unilateral or bilateral tumour. In 60% of cases disease is Unilateral and of these cases 15% are hereditary. All bilateral and multifocal cases are hereditary.

Retinoblastoma can develop as a result of a somatic or germinal mutation, but the susceptibility to the disease is inherited in an autosomal dominant manner. Kundson's[1] "Two Hit Hypothesis" provides an explanation for the genetic basis of two forms of retinoblastoma: (i) the heritable, which is bilateral and multifocal, and (ii) the non-heritable, which is unilateral and unifocal. Stallard[2] gave the first clue to the location of retinoblastoma gene in 1962. High-resolution banding localised the retinoblastoma gene to sub-band 13q14.11. Only 5-6% of patients with retinoblastoma have cytogenetic deletions; the remaining 95% of the mutations occurring in the retinoblastoma gene are submicroscopic.

Patients with genomic 13q14 deletions could pass the susceptibility to retinoblastoma to 50% of their offspring. Genetic testing is crucial for accurate risk prediction for retinoblastoma in close relatives of probands. Of the genetic tests available, karyotype study can be performed in basic genetic testing laboratory. The present study, conducted at a Genetic laboratory, B.J. Medical college, Ahmedabad, Gujarat, we propose to detect the cytogenetic aberrations in 25 Retinoblastoma patients.

MATERIAL AND METHODS

In present study, 25 Retinoblastoma patients from Ophthalmology Outpatient Department (OPD) and admitted in ophthalmology ward , Civil Hospital, Ahmedabad were selected. Karyotypic study was done

in Genetic Laboratory , anatomy department ,B.J.medical college, Ahmedabad. A prior written consent was taken from the parents of these patients.

Amount of 0.5 c.c. of heparinised whole blood was innoculated into 5 c.c. prepared tissue culture medium. This was incubated at 37°C for 72 hours. At 70th hour colchicine 0.5 c.c. was added to the prepared tissue culture medium. Harvesting of cultures and preparations of slides was done according to standard procedures. Metaphases were analysed in every case. A modification of the trypsin and Giemsa banding technique of Seabright[3] is used, in which air dried slides are exposed to trypsin solution rinsed in buffer, stained with Giemsa and examined. Treatment with 0.125% trypsin for 20 seconds results in well defined banding patterns.

The metaphases were first scanned under low power magnification to select well-spread and optimally stained metaphases with elongated chromosomes. Good quality metaphases were karyotyped for each patient under 100X objective and additional metaphases, if necessary. Chromosome identification and nomenclature was done according to the International system for Human Cytogenetic Nomenclature. and finally, Karyotype was prepared using conventional cut and paste technique. Correlation of chromosomal findings was done with other Clinical parameters.

RESULT

In present study, Karyotypic study was done in 25 Retinoblastoma patients.

Table 1: Sex distribution & Eyes affected in patients

	Bilateral	Unilateral		Total
		Rt. Eye	Lt. Eye	
Male	4	5	3	12 (48%)
Female	4	7	2	13 (52%)

Out of 25 patients; 12(48%) were male patients and 13(52%) were female patients. Numbers of patients presented with unilateral retinoblastoma were 17(68%) and bilateral retinoblastoma were 8(32%). Male: female ratio is almost equal and unilateral disease is significantly high.

Table- 3: Karyotypic analysis in present study

Metaphase findings	Females	Males	Total
Numerical abnormality	0	0	0
Structural abnormality	1	0	1 (4%)
Normal	12	12	19 (76%)
Total	13	12	25

Out of 25 patients studied; disease was sporadic with negative family history of retinoblastoma in 16(64%) patients and families with positive family history of retinoblastoma in 9(36%) cases.

The relative incidence of sporadic retinoblastoma is more as compared to cases with positive family history (familial).

In present study out of 25 individuals; 12 Males and 13 Females were studied for cytogenetic assessment. Out of them one female (4%) had 13q14 deletion. 24 (96%) showed normal chromosomal constitution.

DISCUSSION

The chromosomal abnormality found in retinoblastoma cases is deletion of 13q14. Karyotype analysis should be interpreted by taking into consideration the technical limitations like extent of elongation of the chromosomes, variation in trypsinisation and staining intensity of the chromosomal segments and the number of metaphses with 13q14 .In present study in 24 cases ,the declared normal karyotypes by conventional karyotypic study, require confirmation by more specific techniques like Fluorescence in situ hybridization (FISH) and/or CGH (Comparative genomic hybridization) to find out any molecular level anomaly.

As shown in table- 1, in present study, out of 25 patients studied; 12(48%) were male patients and 13(52%) were female patients. 17(68%) patients had unilateral retinoblastoma and 8(32%) patients had bilateral retinoblastoma. So, in our study male: female ratio is almost equal and unilateral disease is significantly high. Shanti pada das[4] et al, LIU XI-xian[5] et al, Harini[7] et al, showed Retinoblastoma affects both boys and girls equally and most of the cases are unilateral. present study is well correlated with these studies.

Table 4: Comparison of present study with other workers

Study	Retinoblastoma patients	13q14 deletion	Familial	Sporadic
LIU XI-xian[5], et al.	8	2 (25%)	1 (12.5%)	7 (87.5%)
K.Srinivasa Rao[6] et al.	8	2 (25%)	0	8 (100%)
Rajasekhar Harini[7] et al.	81	6 (7.40%)	8	73
Kyra Michalova[8] et al.	8	2 (25%)	1 (12.5%)	7 (87.5%)
Bojinova RI[9] et al.	228	13 (5.7%)	-	-
Biju Joseph[10] et al.	58	7 (12%)	-	-
Present study	25	1 (4%)	9 (36%)	16 (64%)

In present study one female (4%) had 13q14 deletion (figure-1). Other Studies have reported 6-25% 13q14 deletion in retinoblastoma patients (table-4). In our study 16(64%) patients had negative family history of retinoblastoma (sporadic) and 9(36%) had positive family history of retinoblastoma. (Table – 2) The incidence of sporadic retinoblastoma is more as compared to cases with positive family history that is well correlated with other studies (table-4).

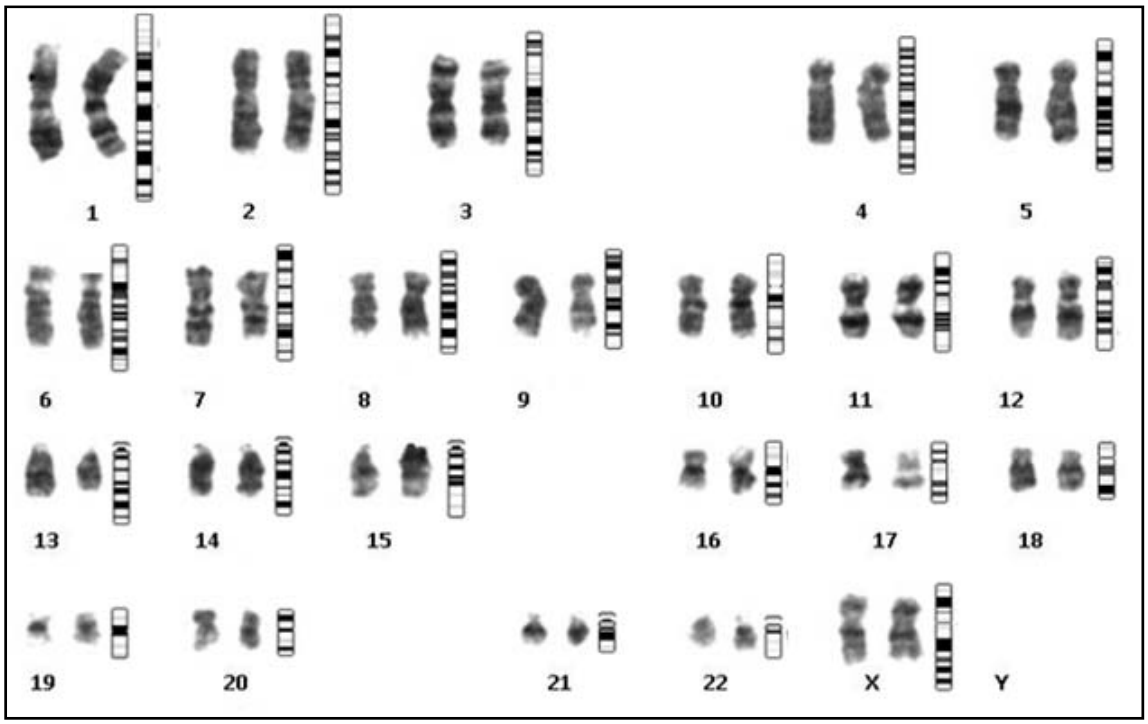

Fig 1: Karyotype of a female having 13q14 deletion

CONCLUSION

In retinoblastoma genetic screening, karyotype analysis is the simplest test providing results within few days of receipt of blood sample. It is the affordable test to most of the retinoblastoma families. Other advantages of karyotype analysis are, easy to master, stable characteristics of the chromosomes with clearly defined abnormal states, possibility of preservation of slides for future applications (mounted slides or in fixative), detection of multiple chromosomal abnormalities from a single specimen, interpretation of results without simultaneous parental testing (required in molecular cytogenetic deletion analysis) and quality of culture could be assessed during incubation and subsequent processing of the blood specimen by naked eye examination. In present study karyotypic analysis of retinoblastoma cases was done to confirm the diagnosis, to predict severity of condition and to counsel the families for the recurrence. In present study, male: female ratio of retinoblastoma patients is almost equal, unilateral disease is significantly high, one female (4%) had 13q14 deletion (figure-1) and incidence of sporadic retinoblastoma is more as compared to cases with positive family history. Genetic testing is crucial for accurate risk prediction for retinoblastoma in close relatives of probands.

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