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THE IMPORTANCE OF THE STUDY OF RADIOSENSITIVITY IN HUMAN GENETIC DISORDERS

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ABSTRACT

It is a well established radiobiological concept that no radiation doses can be considered completely safe. Radiobiologists have been struggling to estimate the health risks from low doses of radiation in human beings for decades. Little is known on the underlying predisposition of the individuals so affected. Hence in this study few established precancerous chromosomal syndromes were subjected to radiation 1) to check for the existence of increased radiosensitivity as measured by chromosomal endpoint, 2) if any syndromes with high radiosensitivity are non tumorigenic and 3) whether radiosensitivity is a prerequisite for the induction of cancer. Our results showed that the radiosensitivity of the chromosomes is significantly greater both in patients and their relatives of high myopia than the normal. Further, the type of chromosomes that are more frequently involved in the production of break points than those of other chromosomes vary in different human genetic disorders studied.

KEYWORDS **Radiosensitivity, human genetic disorders, precancerous chromosomal syndromes, cytogenetic analysis**

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INTRODUCTION

Greater interest in radiosensitivity stemmed from the fact that in a large number of pediatric chemo and radiotherapy trials the proportion of patients as high as 10-15% is usually reported having severe treatment related toxicity occasionally resulting in toxic death. Many studies showed the incidence of increased radiosensitive individuals in the general population. [1] It was reported that about 5% of normal population and 9% of healthy controls were radiosensitive[2]. It was demonstrated that there exists variability in response to radiation in normal individuals[3]. Several hereditary disorders including immunodeficiency (ID) syndrome or repair disorders such as AT, Nijmegen breakage syndrome (NBS) were associated with an elevated risk for severe radiotherapy related toxicity. These cases highlight that severe treatment related complications in pediatric cancer patients may be a result of increased intrinsic radiosensitivity in NBS, AT, and other ID syndromes. Hence, the identification of radiosensitivity sub groups in human population is of considerable societal importance and is of interest and concern to the low dose radiation programme. Further, if people with genetic disorders have increased radiosensitivities, its determination can have implication for further radiotherapy in these individuals. Hence, a study to screen for the high radiosensitivity syndrome is undertaken. Further it is well known that several chromosomal syndromes such as Down, Turner and Klinefelter, as well as several chromosomal instability syndromes such as Ataxia Talangiectasia (AT) Bloom syndrome(BS) and Fanconi anemia (FA) that are prone to cancer (Precancerous syndrome) also showed high radiosensitivity, which created a strong interest to screen for the high radiosensitivity group of genetic disorders that may also have a bearing on cancer induction and to understand whether radiosensitivity is a prerequisite for the precancerous syndromes to attain cancerous state and on the other hand to check, do all the precancerous syndromes possess high radiosensitivity.

Hence, the object of this project is 1) to subject few new established precancerous chromosomal syndromes to radiation to check for the existence of increased radiosensitivity as measured by chromosomal endpoint, 2) do any syndromes with high radiosensitivity are non tumorigenic and 3) to enquire whether radiosensitivity is a prerequisite for the induction of cancer.

MATERIALS AND METHODS

All blood samples were obtained with informed consent and the study was approved by the University ethical committee.

The first part of the study is to establish the high radiosensitivity in precancerous chromosomal syndromes, Two syndromes namely- gonadal dysgenesis with 26 cases, Turner with 10 cases were selected. For the second part, to screen for high radiosensitivity and to detect any association with cancer / precancer – retinitis pigmentosa (RP) with 5 cases, high myopia with 5 affected and 2 unaffected and for the third part of our study, the precancerous chromosomal instability syndromes namely- xeroderma pigmentosa with 6 cases were selected.

Information regarding parental age, parental consanguinity, birth order of the proband, familial incidence of the anomaly if any, reproductive history of the mother, congenital anomalies among the sibs of the probands and other associated disorders of the probands were recorded for further genetic studies.

Culture method

Heparinized blood was collected after venous puncture. Chromosome preparations from the lymphocyte cultures for karyotypic analyses were made according to the standardized procedures in the Biomedical Genetics Research Lab at VIT University[4].

Irradiation of leukocyte culture and chromosome preparation method

The radiosensitivity of the lymphocyte chromosomes of the patients and control individual was assessed by exposing the blood samples to a predetermined suitable dose of 50 rad of gamma rays from a Janus cobalt source at G2. For each patient/control, two culture vials [each vial containing 0.5 ml of blood, 5 ml of culture medium (McCoy's 5a, Sigma), 1 ml of human AB serum and 0.2 ml of phytohemagglutinin (PHA)] were irradiated. The metaphases were arrested with the addition of colchicine (Sigma, 0.001%) at 72 h. Cells were subjected to hypotonic treatment (0.075 M KCl, Merck) and fixed in methanol:acetic acid (3:1), slides were made.

Analysis of chromosomal aberrations

A thorough cytogenetic analysis was carried out. For each individual, a minimum of 50 Giemsa stained or banded metaphases were scored by direct microscopic analysis and by photography wherever needed. Various structural abnormalities such as – chromatid breaks and deletions, the type of chromosome involved and the location of the break points were simultaneously recorded. The radiosensitivity is expressed in total chromosome aberrations per cell. The results of the cytogenetic analysis are presented in Tables-1 to 6.

Analysis of radiosensitivity of individual chromosomes and their regions

The radiosensitivity of every chromosome type was calculated by dividing the number of break points observed on that type of chromosome by the total number of break points observed on the whole genome. From this, statistically significant highly radiosensitive (hot spotted) chromosomes were identified for each syndrome[4]. The pattern of break point distribution was noted.

In the same way increased radiosensitivity of a region (hot spot) if any, on the hyper radiosensitive chromosome was calculated by dividing the number of break points observed on a hot spot region by the total number of break points observed on the whole hot spotted chromosome and expressed in percentage.

RESULTS

1. ***Radiosensitivity of the genomes of the tumorigenic and non tumorigenic syndromes:***

Part I Chromosomal precancerous syndromes

a. Gonadal dysgenesis (GD)

The results of radiosensitivity of 26 gonadal dysgenesis patients are presented in Table 1. Radiosensitivity of the chromosomes is greater in patients of gonadal dysgenesis (0.95±0.08) than the normal (0.58±0.06). The metaphase analyses showed an increased frequency of break points in chromosome 7 and X in gonadal dysgenesis (Table 6).

b. Turner Syndrome

The results of radiosensitivity of 10 Turner patients are presented in Table 1. The mean number of aberrations/cell in 45,XO cells (0.96±0.52) is greater than that found in normal (0.58±0.06). The metaphase analyses showed an increased frequency of break points in chromosome 22 in Turner (Table 6).

Part II Non tumorigenic syndromes with high radiosensitivity:

a. High myopia (HM)

The radiosensitivity of 5 high myopia patients and 2 of the unaffected relative of them are presented in Tables 3 and 5.

The radiosensitivity of the chromosomes is significantly greater both in patients and their relatives of high myopia than the normal.

b. Retinitis pigmentosa (RP)

The results of radiosensitivity of 5 RP patients are presented in Table 3. A significantly high

mean radiosensitivity of RP on the basis of 5 patients is 1.45±1.76 per cell when compared to the control data of 0.58±0.06 per cell. The metaphase analyses showed a significantly increased frequency of radiation induced break points in chromosomes 3, 17, 18, 20 and 22 than that could be expected on the basis of their length based on their DNA content. (p value 0.01, 0.01, 0.05, 0.001 and 0.02 respectively) (Table 6).

Part III Precancerous chromosomal instability syndromes

a. Xeroderma pigmentosa (XP)

The radiosensitivity of six XP patients are presented in Tables 2 and 4. Radiosensitivity of the chromosomes in patients of XP was 0.38±27.01 as compared to that of the normal (0.58±0.06). The metaphase analyses showed an increased frequency of break points in chromosome 1, 2, 3, 4 and the X in XP than that could be expected on the basis of their length or on their DNA content (Table 6). Our results further demonstrate that the type of chromosomes that were more frequently involved in the production of break points than those of other chromosomes vary in different human genetic disorders studied.

Table 1
Radiosensitivity of precancerous chromosomal disorders

S.No	Syndromes	No of metaphases analysed	Mean±SD
1	**Control (n=10)**	**500**	**0.58±0.06**
2	**Gonadal dysgenesis (n=26)**	**1300**	**0.95±0.08**
3	**Turner (n=10)**	**500**	**0.96±0.52**

Table 2
Radiosensitivity of non tumorigenic syndrome with high radiosensitivity

S.No	Syndromes	No of metaphases analysed	Mean±SD
1	**Control (n=10)**	**500**	**0.58±0.06**
2	**High Myopia (n=5)**	**250**	**69.42±15.55**
3	**Retinitis pigmentosa (n=5)**	**250**	**1.45±1.76**

Table 3
Radiosensitivity of precancerous chromosomal instability syndrome

S.No	Syndromes	No of metaphases analysed	Mean±SD
1	Control (n=10)	500	0.58±0.06
2	Xeroderma pigmentosa (n=6)	504	0.38±27.01

Table 4
Frequency of chromosomal aberrations in affected members of precancerous chromosomal instability syndrome family

Patients Xeroderma Pigmentosa (XP)	Number of metaphases analysed	Number of chromatid breaks	Number of aberrant cells	Aberration per cell	% aberrant cells
XP-1-1*(proband/F) Family 1	50	5	4	0.1	8
XP-2-1*(proband/F) Family 2	50	4	3	0.08	6
XP-2-2*(son)	100	30	24	0.3	24
XP-2-7*(son)	50	35	13	0.7	26
XP-2-9*(granddaughter)	77	2	2	0.025	2.59
XP-2-12*(son)	86	40	29	0.46	33.72

Table 5
Frequency of chromosomal aberrations in affected and unaffected members of non precancerous syndrome family

Patients High Myopia (HM) and RP-like complaints	Number of metaphases analysed	Number of chromatid breaks	Number of aberrant cells	Aberration per cell	% aberrant cells
HM-1-1*(proband/m)	50	63	27	1.26	54
HM-1-2*(maternal grandmother)	50	80	39	1.6	78
HM-1-3*(mother)	50	85	39	1.7	78
HM-1-6*(maternal uncle)	50	56	35	1.12	70
HM-1-7*(maternal uncle)	50	62	39	1.24	78
HM-1-1*(proband/m)	50	63	27	1.26	54

Table 6
Frequency of radiation induced chromosome break point distribution in precancerous and non precancerous syndromes

Chr. No.	Chromosome Length (DNA content)	Control Obs. BP%	Gonadal Dysgenesis Obs. BP%	Turner Obs. BP%	Retinitis Pigmentosa Obs. BP%	Xeroderma Pigmentosa Obs. BP%
1	8.25	7.58	7.70	9.87	10.34	11.71
2	8.06	6.11	6.86	9.61	1.97	8.33
3	6.67	6.44	7.49	10.68	14.28	5.55
4	6.38	5.08	7.33	8.60	0.49	5.75
5	6.11	6.09	7.84	6.85	3.94	1.19
6	5.77	4.58	8.60	5.65	0.49	0
7	5.29	2.99	11.98	4.28	0.98	2.38
8	4.87	2.23	7.02	2.11	4.92	0.79
9	4.53	4.38	7.13	5.21	1.97	1.19
10	4.45	3.70	5.78	2.72	3.44	0.79
11	4.55	3.47	4,67	1.81	3.94	0
12	4.49	3.00	4.70	4.92	1.97	0.99
13	3.55	2.53	3.33	1.32	1.47	0.39
14	3.44	4.21	5.51	4.68	3.94	2.58
15	3.23	3.76	4.67	2.41	1.47	1.39
16	2.96	3.87	3.78	2.48	5.41	0.59
17	2.85	4.00	4.79	2.78	7.38	0.19
18	2.67	2.39	2.49	0	5.91	1.98
19	2.06	1.87	2.59	0	2.46	0
20	2.29	0.39	2.70	0	9.35	0
21	1.57	1.05	3.85	1.09	1.97	0
22	1.67	0.49	2.07	3.06	4.92	0
X	5.16	3.71	13.37	12.20	6.89	3.17

Chromosome Length is based on its DNA content[11]

Chr No.: Chromosome Number; Obs BP: Observed breakpoints

DISCUSSION

Genetic changes underlying the conversion of cells from the normal to malignant state and their impact on cellular radiosensitivity is a subject of considerable interest. The fact that some human Mendelian disorders have cancer as a sole feature, a frequent concomitant or a rare complication has long been known. A more recent compilation shows that about 350 of the disorders listed in McKusick's 1992 compendium are associated with cancer of the one type or another. Interest in the question of whether the cells of individuals with such disorders would be radiosensitive was catalysed by two important discoveries in the late 1960s. First in 1968 Cleaver demonstrated that in patients with Xeroderma pigmentosum repair is the biochemical cause for cellular UV hypersensitivity which in turn leads to skin cancers induced by solar radiation. Second, it was found that patients with ataxia telangiectasia (AT), another autosomal recessive disorder had a marked reaction to conventional X- ray therapy[5].

The two themes – cancer predisposition and potentially increased sensitivity of the predisposed individuals to cancers induced by ionizing radiation - are now coming into sharper focus in both basic cancer biology and radiation carcinogenesis. There are at least three reasons for this : The discoveries that mutations underlying some of these disorders are in 'tumor suppressor genes' and / or in those involved in the maintenance of genomic stability, cell cycle control and DNA repair have imparted a new dimension to our thinking about cancer predisposition. A view that has gained currency in recent years is that, in addition to the rare mutant genes which confer a lesser degree of risk without obvious familial clustering position may contribute significantly more to the cancer. With regard to radiation, there is some evidence that cancer predisposed individuals may also be more sensitive to prove to be true, the risk of ionizing radiation. Should this prove to be true, the risk of radiation induced cancers in a population in which these radiosensitive subgroups exist may be higher than in a population which does not have these subgroups.

The relationship between cancer predisposition and the sensitivity of individuals predisposed to cancers induced by ionizing radiation is not comprehensive and hence further radiation cytogenetics study with a different type of approach is required.

Hence we have taken up a project to verify and detect certain facts that may help to throw some light on these unsolved and unanswered areas in cancer, cancer prone syndromes and radiosensitivity. Our first experiment was designed to analyse some well established precancerous chromosomal syndromes to establish its radiosensitivity, to confirm the general view that high radiosensitivity associated with precancerous syndromes. We selected two precancerous syndromes namely gonadal dysgenesis which is predisposed to gonadoblastoma and Turner syndrome which is also prone to cancer. The result from our studies confirms that both those syndromes possess very high radiosensitivity. Our findings agree with earlier workers[6] who concluded that the chromosomal radiosensitivity is significantly higher in cells which are precancerous trisomic for the whole or a part of a chromosomes than in cells with balanced type of anomalies and the monosomic cells is found to be at the same level of sensitivity as that of the normal karyotype.

It was estimated that a significantly increased radiation induced chromosomal aberrations in chromosomal breakage syndromes[7]. Similar type of results was obtained by others[8] in Ataxia telangiectasia and in Nijmegen breakage syndrome.

At present, the evidence for increased sensitivity of cancer predisposed genotypes to radiation induced cancers is limited. However, current knowledge of the known functions of the cancer-predisposing genes and of the consequences of mutations in these provide (a) sufficient grounds for assuming that the

genotypes of those predisposed to cancer may be at an increased risk for radiation induced cancers and (b) the rationale for attempts to estimate quantitatively the impact of genotype-dependent differences in cancer predisposition and radiosensitivity on cancer risks in an irradiated population[9].

It was suggested that no marked x-ray sensitivity among cell strains representing a variety of diseases in which chromosomal instability, increased incidence of neoplasia, or precancerous lesions were observed[10]. There are, however, several cases of heterogeneity of radiosensitivity seen among patients with a particular disease. Among patients with progeria, for example, it was observed a range of Do's from 96 to 140. It is possible that in a number of the other diseases represented by single patient, the normal observation was fortuitous. At the cell survival level, this heterogeneity is analogous to the reported response of cell strains derived from XP patients to UV.

The radiosensitivity that is consistently seen in Down syndrome lymphocytes is not observed in skin fibroblasts and therefore does not appear to be a general property of all tissues in individuals with this disease. A satisfactory explanation for this apparent discrepancy is that lymphocytes from individuals with Down syndrome enter DNA synthesis 8h sooner than lymphocytes from unaffected individuals after stimulation with mitogens. The frequency of ionizing radiation induced chromosome aberrations has been shown to increase with time after mitogenic stimulation; therefore, the radiosensitivity in Down syndrome lymphocytes is likely to be due to differences in their growth properties in culture rather than to differences in their response to DNA damage.

In the second part of our study we designed experiments to screen for non tumorigenic syndromes with high radiosensitivity to prove that association of high radiosensitivity in non tumorigenic syndromes.

For the screening we selected retinitis pigmentosa and high myopia patients. These syndromes show a significantly increased radiosensitivity and no evidence for their precancerous nature. In high myopia we came across an interesting and informative observation.

The frequency of radiosensitivity in all family members affected and clinically unaffected with high myopia and RP-like complaints (consisting of 7 members of 1 family) are similar. The mean percentage of aberrant cells per person for the irradiated samples was 69.42 ± 15.55 and statistically significant (0.001 level). None of the seven members showed any sign of precancerous trait. The results indicate that the members of the family were homogenous in their radiosensitivities irrespective of whether they were clinically unaffected or affected. These results further support the finding that the high radiosensitivity observed is not a consequence of the defect present but due to the familial genome sensitivity of the individuals in the family.

In our third part of our study we designed experiments to screen for precancerous syndrome with radiosensitivity comparable with that of control. We selected two xeroderma pigmentosa families one with 5 members all affected and other with one member affected. In all the members the radiosensitivity was less (0.38 aberration per cell) than that of the control (0.58 ± 0.06). This shows clearly that radiosensitivity is not a prerequisite for a cancer prone syndrome.

CONCLUSION

In our present study we did not find any association between radiosensitivity and tumorigenesis. However the identification of radiosensitivity subgroups in human population is of considerable societal importance and is of interest and concern to the low dose radiation programme. Further if people with genetic disorders have increased radiosensitivity, its determination can have greater implication for further radiotherapy in those individuals. Hence

radiosensitivity screening in human genetic disorders is of great importance.

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