Localized Chromosomal Radiosensitivity as a Biomarker in Amenorrhea with Normal Karyotypes

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Background: The frequency of chromosomal breaks and deletions is higher in cases of primary amenorrhea of gonadal dysgenesis (46, XX) followed by Turners. Karyotype studies may be performed in blood or gonadal tissue or peripheral blood.

Objective: To analyze the radiosensitivity of the chromosomes from patients with primary amenorrhea and compare them to their respective control.

Materials and Methods: The patients studied were classified into 3 groups consisting of 12 patients with 45, X complement (Turner’s syndrome), patients with 46, XX karyotype (gonadal dysgenesis) and a control group of 10 age matched healthy female individuals with proven fertility and with normal karyotype.

Results: The relative radio sensitivity of the individual chromosomes was expressed as the ratio between the patients and the control. When the ratios for any specific chromosome is 1.5 or greater are considered as sensitive chromosomes for radiation. Among primary amenorrhea, radiosensitivity of the chromosomes is significantly higher in patients of gonadal dysgenesis when compared (0.95 ± 0.08) with the control (0.58 ± 0.0646). Chromosome 7 and X showed a significantly high number of breaks at 7q22 and Xq22 respectively. Out of a total number of 167 breaks on No.7 and 178 breaks on X, 67 breaks were on 7q22 and 59 were on Xq22 respectively.

Conclusion: Thus, it may be concluded that both the chromosomal variants of Turner syndrome, namely, 45,X and 46,XX gonadal dysgenesis patients are significantly more radiosensitive than the normal controls. This study shows that radiation can be used as a tool to diagnose genetic disorders in man.

Keywords: Primary amenorrhea, Chromosomes, Radiosensitivity.
INTRODUCTION

Amenorrhea or lack of menstrual cycle is one of the prime causes for female infertility and can be either primary or secondary in nature. The incidence however, is considered to be much higher (63.5%) in cases where ovarian failure is associated with primary amenorrhea (PA). Commonly, PA is found among subjects with Turner syndrome XO, XX- and XY-gonadal dysgenesis.

The term pure gonadal dysgenesis has been described for individuals with gonadal dysgenesis having a normal 46, XX or a 46, XY karyotype chromosome complement. Patients belonging to this category have streak gonads with subsequent sexual infantilism but none of the somatic anomalies associated with Turner syndrome. The external genitalia and the streak gonads of patients with normal karyotype are indistinguishable from those individuals who have gonadal dysgenesis with an abnormal chromosomal complement. Majority of the cases in this series showed signs of pure gonadal dysgenesis. All the cases were observed to have normal 46, XX pattern but for two who exhibited 46, XY Karyotype. Both the patients showed lack of estrogen, because of the presence of the streak gonads, consequently the vagina, cervix and uterus remain small or completely absent. The causes for XX gonadal dysgenesis are failure of genital ridge formation, failure of germ cell migration from yolk sac to genital ridge, genetically determined non-disjunction or anaphase lag limited to germ cell. The application of newly developed leukocyte culture techniques for the high resolution banding of human chromosome and the combined application of computer assisted densitometric chromosome analysis systems on “Q” banded and “G” banded chromosome and the semi automated densitometric system on “Q” banded chromosomes have not only permitted to a more precise description of structural abnormalities of human chromosomes but also made the detection of minute deletions and complicated translocations, possible. This ability to recognize minute chromosomal changes has lead to the wider use of cytogenetic studies in new areas as well as to re-examine and reanalyze the old chromosome syndrome to detect finer chromosomal changes associated with genetic disorders.

Chromosomal aberrations are probably instrumental in the etiology of this category of primary amenorrhea. Minute errors or rearrangements of chromosomal materials are, of course, far more difficult to demonstrate than gross chromosomal change. A normal chromosomal complement in a given patient with certain clinical defects does not always mean the chromosome changes have not occurred. In a number of congenital diseases, no obvious chromosome abnormality has been detected by the application of routine cytogenetic techniques. It is most likely that specific genetic regions might have undergone certain modification. Studies examining X-chromosomal deletions have predicted that Xq aberrations within the Xq13 – Xq 27 region can result in premature gonadal failure.

In 1983, Sutherland by applying his newly developed techniques showed a fragile site on the terminal region of the long arm of one of the X chromosomes in fragile X patients. It seems possible to bring out the hidden chromosomal anomalies in a genetically defective syndrome having a seemingly normal karyotype by the application of appropriate cytogenetic technique. Hence in our present study, we selected ionizing radiation technique as our tool to bring out the chromosomal weak spots, if any, hidden on any of the chromosomes of gonadal dysgenesis having a normal karyotype.

Radiation was selected as a tool because it was established by several workers that the chromosomal breaks induced by radiation are non-randomly distributed on the chromosomes of the normal individuals observed a significantly increased number of breaks on the long arm of the “G” group chromosomes. Caspersson et al reported different sensitivity of different parts of one and the same chromosome. Kiuru et al observed a preferential location of breaks on the bands of weak fluorescence; and Lee and Kara showed in their study of in vitro chromosomal radiosensitivity in human chromosomes of normal individuals, and showed that non-random involvement of chromosome 1, 7 and 12, and a significantly large number of breaks in
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the light band regions and also in the terminal segments. Hence, radiation to the chromosome may bring about the weak spots of the karyotype.

**MATERIALS AND METHODS**

**Radiosensitivity of chromosomes of primary amenorrhea**

The patients studied were classified into 3 groups consisting of 12 patients with 45, X complement (Turner’s syndrome), 26 patients with 46, XX karyotype (gonadal dysgenesis) and a control group of 10 age matched healthy female individuals with proven fertility and with normal karyotype.

**XX-gonadal dysgenesis**

The XX type is characterized by primary amenorrhea, female phenotype normal or eunuchoid proportions, underdevelopment of the breast and secondary sexual characteristics, hypoplastic external genitalia and streak gonads. Most individuals with X gonadal dysgenesis are normal in stature; but some clinical features are common with Turner and XX gonadal dysgenesis.

**Culture Method**

About 1.0 ml of peripheral blood samples were obtained from each patient and the control individuals. 0.5 ml of blood sample was inoculated into each of 2 culture vials containing 5.0 ml of growth medium (McCoy’s 5a), 1.0 ml of human AB serum and 0.2 ml of phytohaemoaggultinin. Two vials per subject were irradiated at a dose of 300 rads [Caesa Gammatron machine (Siemens) available at Cancer Institute, Adyar, Madras] at a focus distance of 20 cm and a dose rate of 204.13 R/min. Cultures were incubated for 69½-70 hrs at 37˚C. Metaphase chromosome preparations were obtained and banded.

**Scoring**

In all patients and controls, 50 metaphases were scored by direct microscopic analysis. Various structural abnormalities such as dicentrics, multacentrics, rings, translocations, breaks and deletions were recorded. The chromosomes involved and the location of the break points in each of these aberrations was also simultaneously noted. The total number of aberrations per cell was obtained by adding the number of chromosome deletions and twice the number of two hit aberrations (which includes rings and translocations) and dividing the sum by the number of cells examined.

**Method of estimation of Radiosensitivity of individual chromosomes in syndrome associated with primary amenorrhea.**

Radiosensitivity of the individual chromosomes was computed as follows:

\[
\frac{a}{Xn} \times 10^3
\]

where

- **a** - Number of chromosomal aberrations in one particular chromosome.
- **n** - Total number of chromosomes studied = 2 x no. of metaphases/individuals x no. of individuals studied.
- **X** - The relative length of the chromosomes.

**RESULTS**

**Radiosensitivity of the genomes of the syndromes associated with primary amenorrhea**

Table 1 shows the frequency of chromosomal aberrations in individual chromosome in various categories of primary amenorrhea and the control individuals. Student’s ‘t’ values for the comparison for two sample means are taken. Among primary amenorrheas, radiosensitivity of the chromosomes is significantly higher in patients of gonadal dysgenesis (0.95 ± 0.08) when compared with the control (0.58 ± 0.0646), at P < 0.001 level.

The relative radiosensitivity of the individual chromosomes was expressed as the ratios between the patients and that of the control. The values so computed are shown in Table 2. When the ratios for any specific chromosome are 1.5 or greater, considered as sensitive chromosomes for the radiation. The distribution ratios for the individual chromosomes in each of the category of primary amenorrhea show that Chromosome 20, 21 and
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Table 1: A Comparative Analysis of the Radiosensitivity of individual Chromosomes in Syndrome associated with Primary Amenorrhea

<table>
<thead>
<tr>
<th>Chromosome no.s</th>
<th>Control</th>
<th>46,XX (GD)</th>
<th>XO(Turner)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.58</td>
<td>7.70 (1.0)</td>
<td>8.29 (1.1)</td>
</tr>
<tr>
<td>2</td>
<td>6.11</td>
<td>6.86 (1.1)</td>
<td>8.10 (1.3)</td>
</tr>
<tr>
<td>3</td>
<td>6.44</td>
<td>7.49 (1.2)</td>
<td>8.54 (1.3)</td>
</tr>
<tr>
<td>4</td>
<td>5.08</td>
<td>7.33 (1.4)</td>
<td>6.48 (1.3)</td>
</tr>
<tr>
<td>5</td>
<td>6.09</td>
<td>7.84 (1.3)</td>
<td>6.72 (1.1)</td>
</tr>
<tr>
<td>6</td>
<td>4.58</td>
<td>8.60 (1.9)</td>
<td>5.51 (1.2)</td>
</tr>
<tr>
<td>7</td>
<td>2.99</td>
<td>11.98 (4.0)</td>
<td>4.51 (1.5)</td>
</tr>
<tr>
<td>8</td>
<td>2.23</td>
<td>7.02 (3.1)</td>
<td>3.55 (1.5)</td>
</tr>
<tr>
<td>9</td>
<td>4.38</td>
<td>7.15 (1.6)</td>
<td>6.08 (1.3)</td>
</tr>
<tr>
<td>10</td>
<td>3.70</td>
<td>5.78 (1.5)</td>
<td>4.18 (1.1)</td>
</tr>
<tr>
<td>11</td>
<td>3.47</td>
<td>4.67 (1.3)</td>
<td>3.98 (1.1)</td>
</tr>
<tr>
<td>12</td>
<td>3.00</td>
<td>4.70 (1.5)</td>
<td>4.47 (1.4)</td>
</tr>
<tr>
<td>13</td>
<td>2.53</td>
<td>3.33 (1.3)</td>
<td>3.16 (1.2)</td>
</tr>
<tr>
<td>14</td>
<td>4.21</td>
<td>5.51 (1.3)</td>
<td>6.55 (1.5)</td>
</tr>
<tr>
<td>15</td>
<td>3.76</td>
<td>4.67 (1.2)</td>
<td>4.34 (1.1)</td>
</tr>
<tr>
<td>16</td>
<td>3.87</td>
<td>3.78 (0.9)</td>
<td>3.72 (0.9)</td>
</tr>
<tr>
<td>17</td>
<td>4.00</td>
<td>4.79 (1.1)</td>
<td>4.44 (1.1)</td>
</tr>
<tr>
<td>18</td>
<td>2.39</td>
<td>2.49 (1.0)</td>
<td>2.28 (0.9)</td>
</tr>
<tr>
<td>19</td>
<td>1.87</td>
<td>2.59 (1.3)</td>
<td>1.87 (1.0)</td>
</tr>
<tr>
<td>20</td>
<td>0.39</td>
<td>2.70 (6.9)</td>
<td>1.30 (3.3)</td>
</tr>
<tr>
<td>21</td>
<td>1.05</td>
<td>3.85 (3.6)</td>
<td>1.75 (1.6)</td>
</tr>
<tr>
<td>22</td>
<td>0.49</td>
<td>2.07 (4.2)</td>
<td>0.82 (1.6)</td>
</tr>
<tr>
<td>23</td>
<td>3.71</td>
<td>13.37 (3.6)</td>
<td>4.56 (1.2)</td>
</tr>
</tbody>
</table>

GD - Gonadal Dysgenesis

22 are more radiosensitive as compared to those of the control. X chromosome is radiosensitive in both the gonadal dysgenesis with normal karyotype. With reference to the other chromosomes considerable variation was observed. In primary amenorrhea of gonadal dysgenesis (46, XX), Chromosomes 7, 8 are highly sensitive whereas chromosomes 6, 9, 10, 12 are moderately sensitive. In cases of primary amenorrhea of Turners (45, XO) chromosomes 7, 8, 12, 14 are moderately sensitive. In case of mosaic Turners all other chromosomes are as sensitive as was found in control individuals.

**DISCUSSION**

It has been known that although certain type of chromosomal aberrations both numerical and structural occurs in somatic cells as well as in germinal cells at a low incidence in nature, those aberrations are more frequent upon exposure to ionizing radiation\(^1\). Such induced aberrations are of prime importance on account of their genetic hazards and their significance has been recognized to certain extent, as a subject of both biological and medical interest.

The mechanism associated with X:18 translocation describes a pattern of inheritance, where break points and translocations of the Xq22.3; 18q 23 regions have resulted in variable fertility.

The association between chromosomal congenital anomalies and neoplasia was well documented from the increased incidence of leukemia in Down syndrome, the retinoblastoma in the 13q- syndrome, and gonadoblastoma, seminoma or dysgerminoma in patients with the karyotype of 45, X/ 46, XY mosaicism, Wilm’s tumor in 11q13 deletion syndrome, breast tumor in Klinefelter syndrome\(^12\), Bloom syndrome and Fanconi anemia leading to retinoblastoma. Several hereditary disorders including immuno deficiency syndrome, Nijmegen breakage syndrome were also associated with an elevated risk of cancer\(^13\).

It has been shown that several chromosomal syndromes are predisposed to the development of cancer. Further, the chromosomes of those syndromes have been shown to have greater radiosensitivity than the syndrome having normal karyotypes.

Peripheral blood culture obtained from 49 individuals with various types of chromosomal anomalies (21 trisomy and their variants, 18 trisomy and their variants D trisomy and their variants, B p deletion, 45,X and its variants) and 34 subjects from normal karyotypes were irradiated and the frequency and the nature of chromosomal aberrations were estimated\(^14,15\).

The chromosomal radiosensitivity is significantly higher in cells which are trisomic for a normal karyotype and that the radiosensitivity of the monosomic cells is found to be the same level of sensitivity as that of the normal karyotype\(^16\) estimated a significantly increased radiation induced chromosomal breakage syndromes. Similar types of results...
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were observed\(^{17}\) in ataxia talengiectasia and in Nijmegen breakage syndrome.

The mechanism associated with X: 18 translocation and describe a pattern of inheritance, where break points and translocations of the Xq22.3; 18q 23 regions have resulted in variable fertility.

In general chromosomes of primary amenorrhea with gonadal dysgenesis are more sensitive to the radiation induced genetic damages. It is also interesting to note that the chromosomes of smaller length, viz., 20, 21 and 22 are more prone to radiation damage, than those of longer chromosomes. X chromosomes invariably was found to be radiosensitive in all the categories of primary amenorrhea with the exception of true Turners.\(^{17}\)

It is interesting to note that more number of chromosomes (8, 21, X, 20 and 22) showed a hypersensitivity to radiation in gonadal dysgenesis group than in any other amenorrhea group. Further, chromosome 7 and X showed a significantly high number of breaks at 7q22 and at Xq22 respectively. Out of a total number of 167 breaks on No.7 and 178 breaks on X, 67 breaks were on 7q22 and 59 where on Xq22 (Fig. 1 & 2).

A group of mentally retarded patients showed a normal chromosome compliment until the application of a fragile site detection technique by Sutherland in 1983\(^{18}\). He observed a cytologically weak spot on the X chromosomes of those patients. Thus it seems possible to detect chromosomal defects in genetic syndromes having a seemingly a normal

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Table 2: Comparison of Radiosensitivity of Chromosomes in Primary Amenorrhea with respect to a normal individual

<table>
<thead>
<tr>
<th>Primary Amenorrhea</th>
<th>Relative damage : Patient / Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.5</td>
</tr>
<tr>
<td>Gonadal Dysgenesis</td>
<td></td>
</tr>
<tr>
<td>(46,XX)</td>
<td></td>
</tr>
<tr>
<td>(2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,2,3,4,5, 11,13,14,15, 16,17,18,19</td>
</tr>
<tr>
<td>Turner Syndrome</td>
<td></td>
</tr>
<tr>
<td>(3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,2,3,4,5, 6,9,10,11, 13,15,16,17, 18,19,X</td>
</tr>
</tbody>
</table>

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Figure 1: Radiation induced chromosomal break points in 7 q arm
A,B,D - Break at 7Q 31 locus
C,E,F,G,H & I - Break at 7Q22 locus

Figure 2: A - Radiation induced Xq22 locus, translocation at chromosome no.2 & X
B - Centromere break at chromosome no. X
C- 7q 22 radiation induced locus
D - Radiation induced chromosomal

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karyotype by the application of appropriate cytogenetic techniques\(^{19}\).

Females with an active X in all cells with
the break point not within any functional gene
show a higher incidence (50%) of ovarian failure
(mainly break points within the Xq13-q26
critical region)\(^{20}\).

Hence application of medium dose
(300r) of ionizing radiation (gamma) to the
chromosomes of gonadal dysgenesis having a
normal karyotype of XX brings out any
previously unsuspected chromosomal
rearrangements.

Our results clearly show that the
radiosensitivity of XX gonadal dysgenesis
\((0.9469 \pm 0.0771)\) significantly higher than
normal XX \((0.5800 \pm 0.646)\) are 3-4 times more
sensitive to radiation than the respective
numbers in the control group. Encouraged by the
fact that human chromosomes possess
differential radiosensitivity, attempts were made
to find out the radiosensitivity of genomes of the
chromosomal disorder syndromes\(^{10, 13, 21-25}\).

**CONCLUSION**

Thus, it may be concluded that both the
chromosomal variants of Turner syndrome,
namely, 45,X and 46,XX gonadal dysgenesis
patients are significantly more radiosensitive
than the normal controls. From this type of study
a comparison of the chromosomal
radiosensitivity among the individuals may help
to bring about a better understanding of the
biological mechanism underlying the radiation
in man. It shows that in man there are
differences in radiosensitivity among various
genetic disorders which may shed light on the
relationship between the cell genotype and
radiosensitivity as well as on the possible
mechanisms inherent to the development of
cancer. Above all, radiation can be used as a tool
to diagnose genetic disorders in man.

**ACKNOWLEDGEMENT**

Our thanks are due to Dr. Samundi
Sankari for her help in providing me with
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**CONFLICTS OF INTEREST**

None declared

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