# Cytogenetic Alterations Induced by Radiation in Amniocytes

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## I. INTRODUCTION

When cells are submitted to ionizing radiation, they respond with physico-chemical phenomena like ionization/excitation of atoms and energy distribution over the cells, chemical reactions with formation of free radicals and rupture of chemical bonds and biological alterations leading to specific changes of cellular functions with decrease of cellular activity. Therefore, both tissues and organs are affected through direct and indirect effects. Among these effects, the interaction of radiation with chromosomes is a critical step since they contain the cell's genetic information. [1]

The type of cell determines its sensibility to radiation. The cell radio sensibility increases with the frequency of its division, lower specialization level and with its low differentiation. The cellular radio sensibility also increases with tissue oxygenization, depending on oxygen effect. However, cells use a set of mechanisms called cell rescue able to repair damages caused by ionizing radiation. Due to these mechanisms not all effects are irreversible. The main effects of irradiation depend on the quantity of absorbed dose and the time exposure. They can be classified in acute and chronic effects. The acute effects occur with exposure to high doses leading to reduction of the life expectancy. The chronic effects take place when the organism is exposed to lower doses of radiation during long time periods. Acute exposure is associated to three syndromes that depend on the radiation dose: Hematopoietic, Gastrointestinal and Central Nervous System syndrome. When the organism receives low doses of radiation three effects can occur: somatic, genetic and in-utero. The effects of radiation in DNA lead to alteration of bonds between basis, cross substitution and single or double strand break, causing mitosis inhibition and prevention [1]. The karyotype study is used to identify radiation effects and lesions on chromosomes. Genetic alterations shown in the karyotype can include alterations in the chromosomes number or alterations in chromosomes structure. These chromosomal alterations are visible in metaphase.

In order to evaluate qualitative and quantitative chromosomes alterations that are implicated on the risk of cellular lesions it is extremely important to recognize how the cells respond to X-ray irradiation.

## II. PROCEDURES

### *A. Radiation Procedure*

Cultured amniocytes were exposed to a 4 MeV X radiation produced by *Varian Clinac 600C linear accelerator*. Duplicated cell cultures (A and B) where divided in 3 groups: (i) control (non irradiated), (ii) 3Gy (irradiated for 1m16s at a dose rate of 250 Monitor Units per minute) and (iii) 6Gy (2m32s at the same rate). The irradiation occurred as shown in the figure 1:

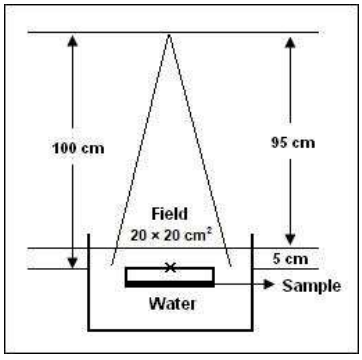


Fig. 1 – Experimental scheme of irradiation.

After the irradiation process, 100µl of colcemid were added to the cultures and incubated at 37ºC for 3h (T0).

### *B. Harvesting*

After the colcemid exposure, culture medium was removed from the cultures flasks and Hank's solution was added to wash the fetal calf serum (FCS). This solution was then discarded and trypsin was added, letting it to act

for 2 minutes at 37ºC, to disrupt cells adherence to the flasks surface. Disaggregation's success was verified with an inverted *NIKON Eclipse TS 100* optic microscope.

Approximately half of the culture flasks' content was transferred to centrifugation tubes and to the remnant of the cells in the flask, new culture medium and supplement were added in order to allow culture reestablishment and propagation. The trypsinized cells were centrifugated for 9 minutes at 1200 rpm. Supernatant was removed. The cells were subjected to hypotonic treatment for 20 minutes with KCl (at 37ºC)

For the pre-fixation, 120µl of acetic acid and methanol in a 1:6 proportion were added to the cells which were then centrifugated for 9 minutes at 1200 rpm. Fixation was achieved by three successive changes of 1:6; 1:3 and 1:1 mixture of acetic acid: methanol. The centrifugation tubes were stored in the refrigerator until slides preparation.

*C. Slide Making*

Cells were spread on to cold glass slides under controlled humidity and temperature conditions. Humidity varies between 40% - 50% and temperature between 18ºC – 20ºC. They were evaluated for the mitotic index using a phase contrast microscope (*Nikon Eclipse E200*).

*D. Banding*

Metaphase spreads were banded with 5% Giemsa in phosphate buffer (pH ≈ 6.8) and studied on a *Nikon Labophoto 2 Microscope*.

Procedures B to D were repeated after 15 hours of the irradiation ($T_1$), 40 hours ($T_2$), 64 hours ($T_3$), 88 hours ($T_4$), 112 hours ($T_5$), 136 hours ($T_6$) and 160 hours ($T_7$).

## III. RESULTS

Morphology and growth of the cells were evaluated on the inverted microscope. Cultures showed a progressive increase of vacuolization over time. In the control it was noticed only after $T_5$, in the 3Gy culture it was observed from $T_4$ and in the 6Gy culture it was shortly after $T_1$ (Table I).

The control culture flasks divided normally until $T_5$. No chromosomal abnormalities were found. After $T_5$ there was an absence of metaphases which coincided with the vacuolization.

In $T_0$ and $T_1$, the cytogenetic study of 3Gy revealed the inexistence of metaphases. In the harvesting times $T_2$, $T_3$ and $T_4$ there was a low mitotic index which decreased in $T_5$ and disappeared in $T_6$ and $T_7$.

In all the 6Gy cultures there were dividing cells but we did not get any phase with chromosomes.

| **Sample** | **Microscope observation after 3h colcemid** | **Results after harvesting** |
|---|---|---|
| Control | Normal amount of cells in division until $T_5$. Slight vacuolization starting after $T_5$. $T_6$ and $T_7$ without dividing cells. | Normal Presence of cells in metaphase until $T_5$. |
| 3Gy | Very few cells in division in $T_0$ and $T_1$ Reduced number of cells with spherical shape (indicative of cell division) in $T_2$, $T_3$ and $T_4$. No cells dividing after $T_5$. Progressive vacuolization starting before $T_5$. | Absence of metaphases in $T_0$, $T_1$ and after $T_5$. Reduced number of metaphases in $T_2$, $T_3$ and $T_4$. |
| 6Gy | Few to absence of dividing cells from $T_0$ to $T_7$. Rapidly increasing vacuolization after $T_1$. | Absence of metaphases. |

Table I – Data related to the inverted microscopic observation after 3 hours of colcemid actuation and the results after harvesting.

Due to low mitotic index, only five metaphases were studied in $T_2$, $T_3$ and $T_4$ harvesting times of both the control and 3Gy cultures.

In the control cultures no chromosome abnormalities were found.

The cells irradiated with 3Gy showed normal chromosomes in $T_2$ and $T_3$; only in $T_4$ there were chromosome break and a fragility in the chromosome 16 (Fig. 2).

Table II – Observations after harvesting.

| Harvesting times | Observations |
|---|---|
| $T_0$ | Absence of metaphases (in the 3Gy and 6Gy culture flasks) |
| $T_1$ | |
| $T_2$ | Normal mitotic index in control culture. Low mitotic index in the 3Gy culture flask. Absence of metaphases in the 6Gy culture flask. |
| $T_3$ | |
| $T_4$ | |
| $T_5$ | Decrease of mitotic index in the control and absence of metaphases in the 3Gy and 6Gy culture flasks. |
| $T_6$ | Absence of metaphases in the control flask, 3Gy and 6Gy. |
| $T_7$ | |

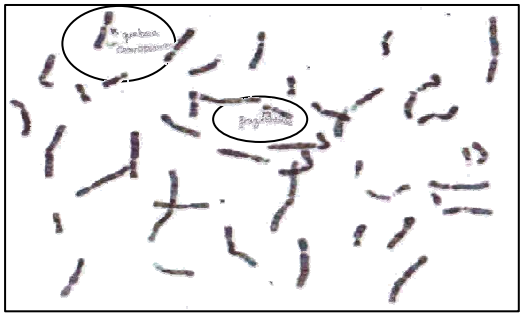

Fig. 2 - Metaphase analysis in harvesting time $T_4$, when cells are irradiated with 3Gy, emphasizing the chromosomal break and fragility in chromosome 16.

## IV. DISCUSSION AND CONCLUSION

In the irradiated cultures the inexistence of metaphases in $T_0$ e $T_1$ (after 4 and 15 hours respectively) is possibly due to the non-recuperation of the cells after irradiation since this was not observed in the control flasks in this period.

In the 3Gy culture flasks metaphases were observed in $T_2$, $T_3$ and $T_4$ (after 40h, 64h and 88h respectively). This is probably due to a resistance of the cells to radiation, that allowed them to recover, responding to the colcemid and producing accountable metaphases; the cell cycle did not seem to be affected between 40 and 88h.

The chromosomes breaks identified in 3Gy $T_4$ culture could be due to the radiation while the fragility seen in chromosome 16 is a documented fact in normal cells which may probably not be related to the radiation [2, 3].

6Gy cultures showed growth but the influence in the cell cycle was such that no metaphases were observed in any of them. This implied that the time of exposure to the colcemid would have to be very different from the one usually done in normal cells in order to achieve metaphases.

Of the present study can be concluded that the main effect of the radiation on the cell culture was the alteration of the time of the cell cycle. This change interfered with the required time of exposure to colcemid to produce metaphases. The repeated culture harvest period led to a progressive vacuolization of the cells which was more evident in the irradiated cultures, particularly in the 6Gy, that showed the vacuolization shortly after the beginning of the experiment.

These results are different from a series done previously (data not shown) which lead us to propose: this experiment should be repeated with more concurrent cultures, so that, the same culture flask would not be harvested so frequently and so we could have a better mitotic index. Different colcemid times have to be experimented in order to find out which ones are most appropriate for irradiated cells and to evaluate the differences between the initial and later exposures. This may be important to determine the effect of the radiation on the biological mechanism of the cell cycle. With better mitotic indexes we can evaluate a greater number of metaphases and therefore have a correct perception of the chromosomal abnormalities caused by radiation.

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