



Original Contribution

COMPARATIVE ANALYSIS OF CHROMOSOME DAMAGE IN TWO GROUPS OF WORKERS HANDLING EITHER RADIOISOTOPES OR ANTI-TUMOUR DRUGS IN AN ONCOLOGY CLINIC OF STARA ZAGORA, BULGARIA

B. Popov^{1*}, V. Petkova¹, S. Georgieva²

¹Department of Molecular Biology, Immunology and Medical Genetics, Faculty of Medicine;

²Department of Genetics, Breeding and Reproduction, Faculty of Agriculture,
Trakia University – Stara Zagora, Bulgaria

ABSTRACT

The aim of the current study was to compare the extent and type of chromosome damage in medical personnel working with ionising radioisotopes and another group working with anti-tumour medications. Two groups of workers from the Interdistrict Dispensary for Oncological Diseases in Stara Zagora were analysed. The first group included 10 people from the Radioisotope Diagnostic Centre in the dispensary, exposed to ionising radiation from different sources for an average duration of 16.4 years. The second group included 6 subjects from the internal ward of the dispensary for oncological diseases, with an average work experience of 14 years, and working with some anti-tumour DNA chelators

The results from the cytogenetic analysis in comparative aspect showed that, while exhibiting varying frequencies, both test groups had chromosome-type and chromatid-type simple aberrations (acentric fragments and chromatid fragments), as well as chromosome exchanges of symmetric and asymmetric types (dicentric). The comparison of the extent of structural damage of chromosomes in both groups showed that the ionising radiation increased the level of asymmetric exchange induction (dicentric chromosomes), while anti-tumour medications (cytostatics) increased the level of acentric fragments induction.

Key words: chromosome aberrations, ionising radiation, anti-tumour medications.

INTRODUCTION

There are groups of people whose professional duties involve being in an environment of increased (over the background) levels of physical and chemical mutagenes. The genetic monitoring of such groups has a health, social, and scientific significance. The purpose is observation and evaluation of the impact of people's contact with various mutagenic factors, and establishing to what extent the mutation danger is increased by the higher levels of these factors. The results and the conclusions derived from the monitoring research help to build up an appropriate system of control and perform adequate action to limit the harmful

impact of environmental mutagenic factors.

Studying mutagenesis in humans is very important since the mutation process is at the root of hereditary diseases and cancer (1, 2). The process can be characterized by its intensity and dynamics – according to the frequency of the emergence of new mutations, or to the ratio of the different types of mutations, which may lead to different end results, or according to the causes – whether it is a spontaneous or induced mutation, e.g. caused by physical and chemical factors. (3, 4, 5).

It has been established that radioisotopes and anti-tumour medications used in medical practice have a clear mutagenic effect (6, 7, 8). The fact that there are groups of people working in an environment of ionising radiation or anti-tumour medications, even though exposed to low levels and concentrations of these factors,

* **Correspondence to:** Borislav Popov,
Department of Molecular Biology, Immunology
and Medical Genetics, Faculty of Medicine,
Trakia University – Stara Zagora 6000, Bulgaria,
E-mail: bnpopov@yahoo.com

which are still above the norm, establishes the necessity of a closer study of these personnel (9, 10).

There is very little data on the quantitative assessment of cytogenetic effects of "extralow" doses, despite the high number of studies on the subject.

Having that in mind, we aimed at determining the extent and types of chromosome damage in a group of medics working in an environment of ionising radiation, and a group working with anti-tumour medications.

MATERIALS AND METHODS

The current study was performed on 2 groups of medics from the Interdistrict Dispensary for Oncological Diseases – Stara Zagora in Bulgaria.

The first group included 10 workers from the Radioisotope Diagnostic Centre of the dispensary, who were exposed to ionising radiation for the duration of 8–19 years, or an average of 16.4 years. These test subjects worked regularly with diagnostic isotopes J^{131} , Tc^{99m} , In^{133m} , Se^{75} , Sr^{85} , Hg^{197} , Hg^{203} . By using dosimeter tools – film dosimeters and ionising dosimeters – these people's average annual dose of irradiation was determined.

The second test group included 6 people from the Internal Ward of the dispensary of oncological diseases, that have worked there for an average of 14 years with the following anti-tumour medications - Novatron, Vincristine, Venazid, Methatrexate, Vinblastine, and Bleomycin.

The control group included 10 people working in a mutagen-free environment.

The samples for measuring the chromosome aberrations were prepared through short-term cultivation of peripheral venous blood lymphocytes by the micromethod of Hungerford (11). Briefly, 1 ml venous blood was obtained from each subject, in a first-stage heparinised single-use syringe, with heparin concentration of 30 IU/ml blood. The cultivation was performed in sterile, brown-coloured, 20 ml borosilicate flasks, containing: 7 ml nutrient medium for cell cultivation RPMI-1640 with L- glutamine and HEPES buffer; 3 ml heat-inactivated calf serum; 0.2 ml re-substituted 2% phytohaemagglutinin; 100 IU/ml penicillin, and 50 mg/ml gentamicin; 0.5 heparinised venous blood. The flasks were put in a sterile box and left in a thermostat at 37° C for 72

hours. On the 70th hour, we added colchicine at a final concentration of 0.5 g/ml. Afterwards, we used the classic protocol for the processing of lymphocyte cultures – 10 min hypotonic processing at room temperature, with 0.075 M KCl and a minimum of 4 fixations with a methanol/acetic acid (ratio of 3:1) fixative. The produced suspensions of lymphocyte cultures were placed on ice-cold glass slides. They were then stained with 4% solution of the Giemsa stain for 8 min. After that, the slides were rinsed with phosphate buffer (pH 7.0) and thoroughly washed with distilled water.

We choose this method for determining chromosome aberrations because of its advantages against other types of cytogenetic tests – simplicity and accessibility of producing the necessary cellular material, together with high density of the cellular population; T-lymphocytes were in the G₀ stage of the cell cycle for 2 years, and had a stable karyotype; in the process of cultivation; they represented a synchronously dividing cell population, especially in the first cycle after the mitogen stimulation; they had a low spontaneous level of chromosome aberrations, and at the same time – a high sensitivity to ionising radiation (12). One of the most important advantages of lymphocyte cultures in radiation genetic studies is that they allow for experimental studies, directly on human cells (13).

The establishment of chromosome aberrations was done via visual analysis under a microscope, in well-stained and spread metaphase plates, containing the full range of 46 chromosomes, according to the recommendation of A. Jha and T. Sharma (14)

RESULTS AND DISCUSSION

The values of the average doses of irradiation of the tested subjects working with radioisotopes are presented on **Table 1**. They ranged from 0.01 Sv to 0.02 Sv. The used dimension [Sv], is named Sievert and represents the mean absorbed dose by tissues, multiplied by the respective radiation quality factor. The maximum allowed dose for such people according to normative documents is 0.5 Sv per year. As apparent from **Table 1**, tested subjects did not receive doses above the maximum allowed one for external irradiation.

Table 1: Types of chromosome aberrations for subjects working with radioisotopes at a different age and job duration

№	Job Duration employment (yrs)	Age years	Annual dose		Analyzed cells	Dicentric	Acentric secondary fragments	Chroma-tid fragments (Break)	Aberrant cells number (%)
			Exter-nal irradiation (Sv)	Absor-bed (Gy)		Number (x 10 ⁻³ per cell)			
1	8	44	0,01	0,11	400	0 (0.00)	0 (0.00)	16 (40.00)	16 (4.0)
2	12	45	0,02	0,14	510	2 (3.92)	2 (3.92)	31 (60.78)	30 (5.88)
3	17	45	0,02	0,13	610	1 (1.63)	2 (3.27)	45 (73.77)	43 (7.05)
4	19	42	0,02	0,14	500	2 (4.00)	2 (4.00)	17 (34.00)	19 (3.9)
5	10	42	0,01	0,13	500	1 (2.00)	3 (6.00)	15 (30.00)	17 (3.4)
6	11	37	0,02	0,11	500	0 (0.00)	2 (4.00)	11 (22.00)	12 (2.4)
7	16	42	0,02	0,16	500	3 (6.00)	2 (4.00)	9 (18.00)	12 (2.4)
8	17	44	0,02	0,11	550	0 (0.00)	0 (0.00)	18 (32.72)	18 (3.27)
9	19	47	0,02	0,11	250	0 (0.00)	0 (0.00)	10 (40.00)	9 (3.6)
10	19	47	0,02	0,11	350	0 (0.00)	0 (0.00)	5 (14.28)	4 (1.14)

Table.1 Types of chromosome aberrations in people working with radioisotopes in the Radioisotope Diagnostic Centre.

Over the course of the cytogenetic analysis of the three groups, we established only some of possible chromosome structural aberrations, and accepted the following classification for studying the preparations:

- Aberrated cells.
- Structural chromosome aberrations – dicentric per cell; acentric pairs of fragments; chromatid fragments (breaks).

Breaking of chromosome structures under the influence of ionising radiation lead to the emergence of aberrations, which are divided into single-strand, dual-strand, and multistrand (15).

Dicentric are dual-strand asymmetric aberrations. They are formed by the fusion of the centric fragments of two chromosomes. The fusion is preceded by breakage of the arms of the respective chromosomes. Very

often, the dicentric chromosome is accompanied by acentric chromosome fragments.

Acentric pairs of chromosome fragments are single-strand aberrations, and are produced by end deletion. Usually, they do not lie together with the chromosome of origin. This is due to the fact that during the processing the chromosome preparations, the hypotonic solution and the other technical procedures influence separately the acentric fragments and the chromosome they originated from.

Chromatid fragments (breaks) are single-strand aberrations, and are produced as a result of the breaking of one chromatid. The resulting fragment lies together with its homologous area of the intact chromatid. A break is defined as a fragment, which lies at an angle against its homologous area of the sister chromatid, aside or along its length.

On **Table 1**, the types of chromosome aberrations in people working with radioisotopes are presented.

Table 2: Types of chromosome aberrations in subjects working with antitumour medications

No.	Employment duration (yrs)	Age (yrs)	Analysed cells	Dicentrics	Acentric secondary fragments	Chromatid fragments (Break)	Aberrant cells (%)
				number ($\times 10^{-3}$ per cell)			
1	12	42	400	0 (0.00)	3 (7.50)	10 (25.00)	12 (3.0)
2	20	54	400	0 (0.00)	7 (17.5)	11 (27.00)	18 (4.0)
3	20	49	400	1 (2.00)	6 (12.0)	20 (40.00)	25 (5.0)
4	6	45	400	0 (0.00)	2 (5.00)	17 (42.00)	16 (4.0)
5	1	23	400	0 (0.00)	6 (15.0)	25 (62.00)	30 (7.5)
6	25	52	400	0 (0.00)	16 (40.0)	18 (45.00)	31 (7.75)

Table 3: Average frequency of the different types of chromosome aberrations in subjects working with radioisotopes or anti-tumour medications

Group	Number of tests	Average age	Analysed cells	Dicentrics number ($\times 10^{-3}$ per cell)	Acentric secondary fragments number ($\times 10^{-3}$ per cell)	Breaks per cell	Aberrant cells (%)
1. Radioisotope Diagnostic Centre	10	43.5	4670	9 (1.76)	13 (2.51)	0.036	3.7
2. Internal Ward	6	44.2	2400	1 (0.41)	40 (16.1)	0.04	5.2
3. Control	10	37.5	2000	1 (0.5)	2 (1.00)	0.03	0.18

Table 3 presents the average frequency of the various types of chromosome aberrations for both tested groups and the control group.

In the group of people working with radioisotopes, among 4670 analysed cells, we discovered 9 dicentrics or 1.76×10^{-3} per cell. In the control group, in 2000 analysed cells, we discovered 1 dicentric, or 0.5×10^{-3} per cell. Apparently, in the first studied group (working with radioisotopes), the number of dicentrics was approximately four times higher than the number in the control group. This significant increase in the levels of dicentrics was a clear indication of the medical personnel's irradiation, despite the fact they had received "extralow" doses for the time period of the study.

In the personnel working with anti-tumour medications, for 2400 analysed metaphase plates, we discovered 1 dicentric, or 0.41×10^{-3} per cell, which was close to the control group's result. The comparison showed that the levels of dicentrics in the group from the Radioisotopes Diagnostic Centre were nearly four times higher, compared to the group working in the Internal Ward of the dispensary.

As for acentric fragments, we received the following results in the different groups. In the medics working with anti-tumour medications, we observed 16.1×10^{-3} acentric

fragments per cell, while for the subjects working with radioisotopes in the Radioisotope Centre, these aberrations were about 2.51×10^{-3} per cell. In the control group, the acentric fragments were 1.00×10^{-3} per cell (see **Table 3**). From a comparative point of view, it was determined that the levels of acentric fragments in the Internal Ward personnel were 6 times higher than the levels of these aberrations in people from the Radioisotope Diagnostic Centre, and 16 times higher than the levels in the control group. These results are in accordance with the fact that, in comparison with ionising radiation, chemical mutagens (including some anti-tumour medications) induced more fragments and less translocations. (16).

Over the course of the cytogenetic analysis we also established a higher frequency of chromatid fragments in both tested groups than in the control group. For the people working with anti-tumour medications (cytostatics), the frequency of chromatid fragments was 0.04, while for the group working with radioisotopes it was 0.036.

As for the number of aberrant cells, we got the following results. There were 5.20% aberrant cells for the personnel working with anti-tumour medications, 3.70% for the medics working with radioisotopes, and

0.18% in the control group.

According to reference data, there are a number of studies by other authors that confirm our results.

Petrova (17) studied the amounts of aberrations in the chromosomes of peripheral blood lymphocytes in subjects, exposed to external irradiation with extralow doses for a period of 17 years. The average yearly dose of irradiation was 0.005-02 Sv and a frequency of aberrations significantly below the control one was established.

Ivanov (18) reported similar results from cytogenetic studies of professionally irradiated subjects, with doses varying between 0.01-0.05 Sv. The levels of aberrant cells he discovered were 6.64%.

During in vivo and in vitro research of the mutagenic effect of various chemical factors of a professional nature, a higher frequency of aberrant cells was also established (19, 20, 21, 22).

CONCLUSIONS

The results showed in a comparative aspect that, despite having different frequencies, the two groups exhibited both simple chromosome chromatid-type aberrations (acentric fragments and chromatid fragments), and chromosome exchanges of symmetric and asymmetric types (dicentric).

In the tested group working with radioisotopes at the Radioisotope Diagnostic Centre of the Interdistrict Dispensary for Oncological Diseases – Stara Zagora in Bulgaria, ionising radiation induced a high frequency of chromosome aberrations – dicentric, acentric fragments, and breaks.

In the group working with anti-tumour medications (cytostatics) at the Internal Ward of the Interdistrict Dispensary for Oncological Diseases – Stara Zagora in Bulgaria, a high level of structural chromosome damage was established – acentric fragments, breaks, and dicentric, with the highest frequency belonging to acentric fragments.

Comparing the levels of structural malformations of chromosomes for both tested groups, we determined that:

- Ionising radiation increased the levels of induced asymmetric exchanges (dicentric chromosomes);
- Anti-tumour medications (cytostatics) increased the levels of induced acentric fragments.

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