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ORIGINAL ARTICLE

Chromosomal aberrations after exposure to low doses of ionizing radiation

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Summary

Purpose: *To compare the incidence of chromosomal aberrations (CA) in healthy medical workers occupationally exposed to ionizing radiation (IR) and in non-exposed healthy population.*

Methods: *This was a 4-year study with 462 subjects, mean age 42.3 years, occupationally exposed to IR (exposed group - E), and 95 subjects, mean age 35.2 years, not exposed to IR (control group - C), during the same time period and from the same territory. Thermoluminescence dosimeters (TLD) were used for assessment of IR exposure. Modified Moorhead's micro method for peripheral blood lymphocytes and conventional cytogenetic technique of CA was used for analysis of CA. The karyotype of 200 lymphocytes in metaphase was analysed by immersion light microscope.*

Results: *The average annual absorbed dose measured by TLD was 14.5 mSv in group E and 2.8 mSv in group C exposed to natural level of radioactivity. The incidence of CA was 21.6% in group E and 2.1% in group C ($p < 0.05$), while non-specific chromosomal lesions (gaps, breaks, elongations) were equal in both groups (22%). In group E, the highest incidence was found in nuclear medicine workers (42.6%), then in orthopedic surgeons (27.08%). Highly significant difference ($p < 0.001$) was found in the number of aberrant cells and the sum of CA between group E and C. The sum of CA and the number of aberrant cells were positively correlated with the duration of exposure ($p < 0.001$), and to a lesser degree with age ($p < 0.05$) in group E. In group C, this correlation was negative and insignificant. In group E, subjects with duration of occupational exposure (DOE) up to 15 years (subgroup E I=327) had significantly less number of aberrant cells and CA in comparison with the subjects with DOE over 15 years (subgroup E II=135) ($p < 0.01$).*

Conclusion: *Long-term occupational exposure to low doses IR contributes to the development and increased frequency of specific CA (like dicentrics), but varies in relation to different working places. The majority of subjects had no other genetic modifications (non-specific chromosomal lesions) affected by low doses of IR.*

Key words: chromosomal aberrations, ionizing radiation

Introduction

Deposition of IR energy in genetic material induces the development of stable and unstable structural CA in equal proportion [1,2]. The unstable ones are dicentric, polycentric, ring and terminal deletion, followed by acentric fragments. The aberrant cell may survive 10 divisions at most [2-4]. However, acentric fragments do not follow the inversions and translocations and they may survive in tissue for a long time, representing the constant imbalance of karyotype [5,6]. Erroneous repair processes (misrepair or misreplication) play a major role for the development of CA, transforming the primary lesions of DNA chains into the secondary ones with characteristic forms of aberrant chromosomes [7,8]. The structural aberrations may occur in G1 phase of the cell cycle (chromosome) and in S phase (chromatic) [9]. Analysis of CA is extremely important since it represents a reliable biomarker of radiation effect [10-14].

One of the sequels of deposition of energy during IR of different linear energy transfer (LET) is the distribution of CA in peripheral blood lymphocytes [3-6]. The absorbed dose of IR is determined on the basis of the frequency of lymphocyte aberrations [10,11].

In occupational exposure, that is the exposure to

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low doses of IR, the measurement of the absorbed dose is based on the biological effects to the genetic material [12-14]. The presence of at least one dicentric in 200 tested metaphases is evidence of higher absorbed dose of IR, namely the evidence of exceeding the maximal tolerated dose (MTD) which is 20 mSv for occupationally exposed persons. Calibration curves are used for accurate estimation of high doses [10].

Methods

This 4-year study included 462 subjects, mean age 42.3 years, who were occupationally exposed to low doses of IR, and 95 subjects, mean age 35.2 years, who were not exposed to IR, during the same time period and from the same territory.

The occupationally exposed subjects comprised the exposed group (group E) and included medical workers of various professions from different working places within the area of IR in the public health system: radiologists (n=93) exposed to X-ray; radiology technicians (n=274) exposed to X-ray; physicians and laboratory technicians - workers in isotopic laboratories of nuclear medicine (NM) (n=47) exposed to gamma and beta rays; orthopedic surgeons, cardiologists, anesthetists and others exposed to direct beam of X-ray, workers in interventional radiology (n=48).

All of them possessed personal TLD which were read by appropriate scanner. TDL absorbs the radiation falling on the body (and the dosimeter) during the operation with the radiation sources. The average annual absorbed doses for the exposure period indicate the dynamics of the absorbed dose at the working place in the controlled professional conditions, when it must meet the defined MTD (20 mSv).

The subjects not occupationally exposed to IR formed the control group C and did not possess TLD.

On the basis of the history and general clinical examination defined by the law on protection from IR in our country, complying with the International Commission on Radiological Protection (ICRP) recommendation, all subjects were healthy and were not exposed to genotoxic agents at the time of CA test [13,14].

For analysis of CA the modified Moorhead's micro method for peripheral blood lymphocytes and conventional cytogenetic technique of CA analysis were used. Venous blood (1 cm^3) for lymphocyte culture was taken, poured into sterile heparinized test tubes and stored for 24 h at 4° C.

Culture was performed in RPMI medium with the addition of 0.1% phytohemagglutinin; 2-3 h before the time of culture was up, 0.2 ml of colcemide was added, enabling the transition of cells from metaphase to anaphase, interfering with the function of mitotic spindle [9].

Stained preparations (Giemsa) were evaluated with immersion light microscope. The karyotype of 200 lymphocytes in metaphase was analysed. The most characteristic aberration that is observed is dicentric, then the ring, acentric fragment, pericentric inversion and translocation.

Chromosomal lesions (gaps), e.g., breaks, modifications, and elongations are not characteristic of IR effect only, but they also occur as a result of toxic, infectious, pharmacological and other factors from the working and living environment [12-14], and therefore, being non-specific, they have been designated as findings within normal range.

Statistical analysis

For statistical analysis Student's *t*-test and Student's pair *t*-test were used for processing the clinical-laboratory results and dosimeters data. Mann-Whitney's test of the rank sums was used to compare differences between two groups of non-parametric data. Pearson's x^2 test, in the form of contingency tables, was used for the analysis of CA. For identification of correlation, a single linear correlation and multiple regression analysis were used.

Results

The average annual absorbed dose measured by TLD was 10.5 mSv in group E. In group C subjects, who were exposed to natural level of radioactivity, the average annual absorbed dose for the population of Serbia was 2.8 mSv ($p < 0.05$).

Increased incidence of CA was found in 21.6% subjects of group E and in 2.1% in group C ($p < 0.05$), while non-specific chromosomal lesions were equal in both groups (22%; $p > 0.05$).

Dicentric forms were the most prevailing (12.53%), while stable structural aberrations (translocations and inversions) were found in 6.26% of group E subjects ($p < 0.05$). Among all CA, the unstable ones accounted for two-thirds or 74.6%, out of which 57% were dicentric and only 5.27% were ring CA.

Among occupationally exposed medical workers, the highest incidence of CA was found in nuclear medicine workers (Table 1), then in orthopedic surgeons, cardiologists, anesthetists, and other professionals in interventional radiology, who are generally exposed to direct X-ray beam during their activities.

Table 1. Incidence of chromosomal aberrations in relation to profession

Profession	*Number of examined subjects*	*With CA n (%)*	*Without CA n (%)*
Radiologists	93	16 (17.20)	77 (82.80)
Radiological technicians	274	51 (18.60)	223 (81.40)
Workers in nuclear medicine	47	20 (42.60)	27 (57.40)
Workers in interventional radiology	48	13 (27.08)	35 (72.92)
Total	462	100 (21.40)	362 (78.60)

CA: chromosomal aberrations

There was a highly significant difference in the number of aberrant cells and the sum of CA between the exposed and control groups, which could be expected considering the exposure to IR as the cause of genetic material transformations (Table 2).

However, IR doses were small and could not challenge CA. Long-term occupational exposure and accumulation of small doses led to the creation of CA. In the control group, exposure to natural radiation level was not sufficient for the occurrence of CA, regardless of time.

Nevertheless, over half (56.8%) of group E subjects had no CA.

Group E subjects with DOE up to 15 years (exposed group I, n=327) had significantly less number of aberrant cells and CA in comparison to group E subjects (exposed group II, n=135) with longer DOE (over 15 years; $p < 0.01$; Table 3).

The sum of CA and the number of aberrant cells was positively correlated with DOE ($p < 0.001$; Table 4), and with age ($p < 0.05$) in the exposed workers (Table 5).

In the control group C this correlation was negative and insignificant (Table 6).

Less number of aberrant cells was found in subjects over 40 years of age.

There was no correlation between the incidence of CA and the absorbed doses measured by TLD ($p > 0.05$). TLD doses ranged greatly, from 1.5 mSv to 19.2 mSv (10.5 mSv on average), but this distribution within low doses, below 20 mSv, was not significant.

Table 2. The difference of the aberrant cell number and the sum of chromosomal aberrations between the exposed and control groups

Groups	*Number of subjects*	*Number of aberrant cells x±SD*	*Sum of CA x±SD*
Exposed group	462	0.52±0.03	0.70±0.05
Control group	95	0.24±0.04	0.24±0.05
T-test; p-value		3.83; p=0.0001	4.27; p=0.0001

x: median, SD: standard deviation, CA: chromosomal aberrations

Table 3. The difference of the aberrant cell number and the sum of chromosomal aberrations between the two exposed subgroups

Exposed subgroups of workers	*DOE (years)*	*Number of subjects*	*Number of aberrant cells x±SD*	*Sum of CA x±SD*
Exposed group I	1-15	327	0.46±0.03	0.63±0.05
Exposed group II	16-40	135	0.66±0.07	0.90±0.1
p-value			0.002	0.01

DOE: duration of occupational exposure, x: median, SD: standard deviation, CA: chromosomal aberrations

Table 4. Correlation between the sum of chromosomal aberrations and the aberrant cell number, and duration of occupational exposure

DOE; sum of CA Correlation	*DOE; number of aberrant cells Correlation*
r = 0.128	r = 0.121
p = 0.006	p = 0.009

DOE: duration of occupational exposure, r: coefficient of correlation, CA: chromosomal aberrations

Table 5. Correlation of age with the sum of chromosomal aberrations and the aberrant cell number in the exposed group

Age; sum of CA Correlation	*Age; number of aberrant cells Correlation*
r = 0.110	r = 0.097
p = 0.018	p = 0.038

r: coefficient of correlation, CA: chromosomal aberrations

Table 6. Correlation of age with the sum of chromosomal aberrations and the aberrant cell number in the control group

Age; sum of CA Correlation	*Age; number of aberrant cells Correlation*
r=-0.001	r=-0.060
p=0.993	p=0.565

r: coefficient of correlation, CA: chromosomal aberrations

Discussion

According to the relevant literature and in concordance with our study, low doses of IR lead to noticeable lesions of chromosomes in lymphocytes [12-14]. The radiation workers employed in hospitals, exposed to X-rays at doses lower to the maximal tolerated limits, manifested chromosomal lesions.

Dicentrics, occurring due to double break of DNA

chain in two different chromosomes is too big lesion, rarely found in healthy population (only one case was reported in our controls). It is used to determine the absorbed dose of the IR and is the indicator of the absorbed dose under occupational exposure conditions.

Non-specific chromosomal changes cannot be an indicator of the absorbed dose because they were also found in the same proportion in the general population. For these chromosomal changes it may be that smoking, age, drugs, additives, diet, which can not cause double break of DNA and characteristic CA could be responsible. Non-specific lesions may be caused by different toxic elements if they are united and operate for a long time [12].

Nuclear medicine workers work in laboratories and perform diagnostic procedures with radioisotopes, open sources, and, therefore, there is always a risk for internal contamination [8]. Unfortunately, regular measurement of urine radioactivity is not possible in our conditions in Serbia, what would be the actual evidence of internal contamination considering the use of short-lived radionuclides having short acting time of half-excretion (e.g., hippuran-j131 - only 2.5 days). The annual monitoring of 24 h urine radioactivity revealed significantly lower values in relation to ALI (annual limit of intake), ranging from 0.1 to 10 Bq/l. However, the taken radionuclides (^{131}I, ^{125}I and ^{123}I, technetium $^{99}{}_{m}$Tc and tritium) become the internal source of radiation, especially if an organ depot is present (i.e. thyroid gland for iodine), while in case of equal body distribution (i.e. technetium and tritium, that are evenly distributed in all body tissues) and short retaining in the body, it has also great possibility to act directly to the lymphocyte nucleus causing chromatin ionization and producing radiation-recomposed forms in chromosomes. In case of external radiation, the indirect effect is higher, through free radicals diffusing slowly from the extracellular fluid into the nucleus [3].

Radiologists and radiological technicians are only occasionally in close proximity to the source of radiation in contrast medium radiography and imaging, since they are behind the protective shield or in another room. Moreover, they know best the principles of radiological protection by the nature of their job. Medical workers of other specialties (other than radiology) should not operate x-ray machines, particularly with direct x-ray beam in the interventional radiology.

Higher risk of carcinogenesis in healthy subjects with elevated proportion of CA in lymphocytes has been described in epidemiological studies. Regardless of the role of exposure to carcinogens, the increased risk of cancer in people with higher incidence of aberrant cells is significant [3,13]. This suggests that chromosomal changes in lymphocytes should be recognized as biomarkers of higher probability of carcinogenesis [15-18].

Unstable aberrations that had not been fixed in the cell division were more prevalent in exposed workers, indicating increased absorbed doses of IR in the working places. Since the absorbed doses in the exposed workers are within normal limits and differ rather slightly, differences of results might be due to significant deviation of the individual sensitivity, and especially the efficiency of DNA repair processes, as well as a possible effect of genotoxic environmental agents [19-24].

Healthy subjects, occupationally exposed to IR, may have structural CA, regardless of the low tolerated dose and the correctness of the apparatus and the irradiation source at working places. Most frequently, they are unstable and decline in cell division, so they seem not to have important clinical significance. Nevertheless, some of them may be present in tissues for a longer period of time and be responsible for late sequels [23,24].

Studies have shown that x and gamma rays induce unstable CA in human peripheral blood lymphocytes as a response to the irradiation dose, which is linear in this type of radiation [22-24]. This means that individuals working in interventional radiological methods are the most exposed to the direct bundle of x and gamma radiation as found in our results, as well as in already published articles [15].

The observed dose-response data, when exposure originates from alpha and beta particle radiation (during examinations with radionuclides in laboratories), indicate different distribution of effects over a linear quadratic model, meaning that the higher biodosimetry changes happen in workers of nuclear medicine after contamination, although not proven by usual measurement methods of urine radioactivity [15,19,22]. In cases of exposure to low radiation doses, response correlates with the period of accumulation of effects of these doses during professional exposure [15,23,24].

Within the frame of permissible small doses, no correlations with dose identified from TLD were encountered in our study, coinciding with literature data which point out that increased frequency of CA is a response to total absorbed dose up to 100 mGy or 100 mSv (equivalent dose) [23,24]. TLD-measured dose can be below 100 mGy if the dose had been once acutely absorbed [23] and if the blood had been taken shortly after the accident. In chronic exposure this method has imperfections because of dicentrics lost during the passing of time. On the one hand, small doses are added together and increase the effect, the frequency of CA.

On the other hand, the frequency of CA decreases over time because CA disappear during cell division [23]. Because of that, this method is suitable for chronic professional exposure to IR as a very reliable biomarker for biomonitoring, but insufficient and unreliable for biodosimetry [19-24].

Radiosensitivity tends to decrease with age [2,3].

In our study we found a correlation between CA and age of exposed persons, contrary to the control group and this contrasts prior data. Some researchers claim that there are not correlations with age in exposed or control subjects, while some others maintain that the frequency of DNA changes increases with age [20,21,24].

Although radiosensitivity decreases with age, long-term exposure led to accumulation, and, therefore, the radiobiological effect of the absorbed doses was higher in older subjects with longer periods of exposure.

However, smaller doses are required for persons younger than 40 years to induce unstable CA than in those over 40 years [23]; this was seen also in our investigation and is probably due to higher radiosensitivity of younger persons that have more intensive metabolism.

The significance of personal dosimeters for radiation determination would be higher in case of measurement of the exposure dose and effective equivalent and collective doses, involving - besides TLD - some other parameters such as the source distance and length of time in any individual case, what is actually unfeasible in our conditions in Serbia.

Therefore, the correlation of the frequency of CA and DOE bears important significance due to the cumulative effects of low doses that may not be avoided in continuous occupational exposure.

Cytogenetic biodosimetric investigations complemented with hematologic investigations of exposed workers have helped to completing a national radiation protection program for workers in zones of IR in Serbia [16].

Chromosomal analysis is the method of choice in case of biological dosimeters used for the quantification of exposure to IR.

The dicentric chromosome is characteristic for IR and its spontaneous frequency is very low in the healthy general population (about one dicentric per 1,000 cells). In case of whole-body occupational chronic exposure, doses up to 20 mSv are detectable [23].

On the one hand there is a need for chromosomal analysis because of risk assessments of carcinogenesis and on the other hand because of the relative radiation risk (RR) [16,17]. Contrary to other researchers [17], increase of malignant diseases in workers professionally exposed to IR was not observed, possibly because of insufficient time of the latent monitoring period as well as because of small cohort volume [25,26]. An epidemiological study on 22,358 subjects in 11 countries covering a period of 40 years [17] evidenced linking of frequency and type of CA in peripheral lymphocytes of healthy individuals with early stages of cancer of any localization. The presence of ring chromosomes increased the RR to 2.22. In our subjects ring chromosomes were the most infrequent form.

A high level of CA in peripheral blood lymphocytes may be an early marker of cancer risk, but data on risk of specific cancers and types of CA (chromosome type and chromatid type) are limited [18].

The differences of genetic material modifications under the conditions of exposure may be interpreted by individual features of occupationally exposed subjects that were considered in this study: immunological status, deficiency of DNA repair, genetic instability (predisposition, sensitivity), lack of folates in diet, fatigue, sex, age, etc. [13]. Analysis of CA is necessary as a baseline test of people working in zones of IR. Finding frequent CA in the annual periodical examinations points to an increased health risk [10,17,24-26].

Conclusion

Cytogenetic analysis of the frequencies of CA can be used for evaluating the effects of small doses of IR and the relative risk assessments. This method could be recommended for biomonitoring as a biomarker of IR effects and the working ability of persons working in an area of radiation.

Long-term occupational exposure to low doses of IR contributed to the development and increased frequency of specific CA, but varied in relation with different working places. The majority of subjects had no other genetic modifications affected by low doses of IR.

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