Incidence of spontaneous cytogenetic changes in peripheral blood lymphocytes of a human population sample

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Three mutagenetic methods – conventional structural chromosomal aberration analysis (CA), sister chromatid exchange (SCE) method and micronucleus test (MN) were carried out on a population sample of 350 test subjects aged 18 to 65 years.

The spontaneous incidence of these aberrations in the first in vitro metaphases of lymphocytes was 0.83 % chromatid breaks, 0.43 % isochromatid-chromosome breaks, 0.21 % acentrics fragments and 0.01 % dicentric chromosomes per test subject.

The mean value for SCE per cell amounted to 6.52 ± 0.70, while the frequency of micronuclei was 5.8 ± 2.1 per 500 binuclear lymphocytes. These results represent the mutagenetic background for the Slovenian population and can be used for the assessment or in case of suspect exposure to clastogenic agents.

Key words: Lymphocytes; chromosome aberrations; sister chromatid exchange; micronuclei

Introduction

Intensive industrialisation over the past few decades has resulted in the production and use of numerous genotoxic chemicals and sources of ionising and non-ionising radiation. The need to identify the mutagenic and carcinogenic effects of these agents on the human population exposed environmentally, professionally or accidentally is therefore on the increase. There are several methods with which it is possible to prove changes occurring in DNA molecules. Unfortunately, there are few direct methods for the identification and assessment of the degree of mutations.

Among methods for the detection of large changes in the genome of human somatic cells which are used routinely for mutagenetic monitoring, the International Commission for Protection against Environmental Mutagens and Carcinogens recommends the detection of chromosomal aberrations, the micronucleus test and sister chromatid exchange technique.[1]

The analysis of chromosomal aberrations in peripheral blood lymphocytes has gained the widest use to date. The methodological and technical conditions for this technique and the procedure for the analysis of specimens are well defined, largely owing to the use of specific chromosomal aberrations in biological dosimetry.[2]

It is well known, however, that ionising radiation and the majority of chemical genotoxic agents have different effects on cellular DNA which is directly included in the formation of chromosomal changes.

The frequency of SCE is a sensitive indicator of the effects of chemical genotoxic agents and high linear energy transfer (LET) radiation, but a poor indicator of the exposure to low LET ionising radiation.[3,4]

The micronucleus test has almost universal application in the detection of changes in the cellular genome. Micronuclei may originate from acentric fragments resulting from two-chain breaks of DNA after its exposure to radiation, and have shown very

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good dose-response relationships. They may also contain several chromosomes which were not distributed equally to daughter cells due to a non-functional cell-division spindle or kinetochores. The latter phenomenon is most frequently caused by chemical agents.[5] It therefore seems that all three tests should be used on parallel specimens in order to be able to assess the type of exposure: to a physical or chemical agent, or even possibly simultaneously to both categories of clastogens.[6]

To enable the evaluation and correct performance of cytogenetic population monitoring, it is necessary to know as much as possible about the frequency of normal, spontaneous chromosomal aberrations, as well as about the factors which exert an influence on their occurrence.[7]

Considering the institutions in which the authors of this paper work and the legal obligation of mutagenetic monitoring for defined groups of people who are professionally exposed to ionising radiation, the purpose of this study was:

– to standardise laboratory conditions of in vitro cultivation of lymphocytes in accordance with the Protocol of the International Atomic Energy Agency,

– to determine the mutagenetic background in a population sample of persons who are not professionally exposed to ionising radiation by using the structural chromosomal aberration analysis, micronucleus test and SCE frequency per cell.

Subjects and methods

Subjects

The study included 350 subjects, of whom 153 were students prior to their enrolment at the High School of Radiology, and 197 persons prior to the assumption of their duties in radiation zones. It is important to emphasise that such a selection of the population sample enables comparative analyses regarding mutagenic effects in conjunction with age, and even more, lifestyle factors.

The group of students included secondary school students who had just graduated, aged 18 to 20 years. The majority of them were non-smokers and persons with an extremely low previous influence of alcohol, coffee, drugs and ionising radiation used for diagnostic and treatment purposes.

The second group of test subjects had not been professionally exposed to genotoxic substances before taking of blood samples for mutagenetic tests. However, the age dispersion in this group (20 to 65 years) was significant, and the lifestyles of its subjects had undoubtedly brought along more numerous factors with mutagenic characteristics. At the time when blood samples for mutagenetic tests were taken, none of the test subjects showed any subjective troubles nor objective signs of acute illness, and none of them had been previously subject to major diagnostic or therapeutic irradiation. Test subjects were both men and women, of whom 34 % were smokers.

Methods

Data of subjects was collected by means of filling in Personal Health Questionnaires. Peripheral blood lymphocytes from subjects were used as cellular material. Blood samples were taken simultaneously for all three tests.

Structural chromosomal aberrations (CA)

Standard in vitro lymphocyte cultures were used for structural chromosomal aberration analysis. 0.3 ml of heparinised whole blood was added to 5 ml of the culture medium (GIBCO BRL Chromosome med 1A with Phytohaemaglutinin). The first in vitro cell division cycle was established with an addition of 5 mg/ml of BrdU (Sigma). 0.075 mol/l potassium chloride was used for the hypotonic procedure. Fixation was performed in a mixture of ice acetic acid and methyl alcohol at a ratio of 1:3. The cell suspension was pipetted onto cold glass slides, specimens were air-dried and stained with Giemsa-Sigma. For every test subject, the first 200 in vitro metaphases were analysed at 1000X magnification on a Nikon LABO-PHOT2 microscope. Structural damage to chromosomes was categorised as chromatid breaks, isochromatid-chromosomal breaks, acentric fragments or dicentric and ring chromosomes. Gaps were not included in the total number of chromosomal aberrations.

Sister chromatid exchange (SCE)

The same culture medium was used as for the first test. 72-hour lymphocyte cultures with the addition of 10 mg/ml BrdU were prepared in dark conditions. The procedure was performed according to the Kato (1974) method.[8]

50 cells per subject were analysed, SCE were counted and presented as average numbers per cell. The range of SCE frequencies was also recorded for every subject.

Micronucleus test (MN)

For this test, 3 mg/ml of cytochalasin B (Sigma) was added to each in vitro lymphocyte culture in the 43rd hour of cultivation. The Fenech-Morley (1985) method was used.[9]

Hypotonic procedure was omitted, and specimens were stained according to May-Grünwald and Giemsa. Cells with clearly blocked cytokinesis (CB cells), i.e. binuclear cells, were analysed. 500 cells per person were inspected and the results were presented as the number of micronuclei per 500 CB cells.

Statistical data processing

Data was processed using standard methods of parametric statistics. The differences between the average values for individual groups were tested using the variance analysis method with the SPSS computer program.

Results

Structural chromosomal aberrations (CA) were analyzed in 153 students or 30,600 their first in vitro metaphases, and in 197 technicians pending employment or 39400 their first in vitro metaphases (Table 1). The average value of acentric fragments for technicians pending employment is 0.58 and for students only 0.24. Acentric fragments were found

Table 1. Mutagenetic testing of subjects – Structural chromosomal aberrations.

No.		Tests		% aberrations All subjects		ACENTRICS				
						All subjects			Subjects with acentrics	
	Groups of test subjects	No. of subjects	No. of exam. cells	Mean	SD	Mean	SD	Share of subj. (%)	Mean	SD
0	1	2	3	4	5	6	7	8	9	10
1	Technicians	197	39400	1,812	1,028	0,584	1,045	39,090	1,494	1,199
2	Students	153	30600	1,101	0,580	0,242	0,473	22,880	1,057	0,338
3	Total	350	70000	1,501	0,861	0,434	0,860	32,000	1,357	1,029
	Variance FR			58,647		14,133			4,455	
	analysis FP			0,000		0,000			0,037	

RINGS					DICENTRIC CROMOSOMES					
All subjects			Subjects with rings		All subjects			Subjects with dicentric chrom.	
Mean	SD	Share of subj. (%)	Mean	SD	Mean	SD	Share of subj. (%)	Mean	SD
11	12	13	14	15	16	17	18	19	20
0,005	0,071	0,508	1,000		0,041	0,283	2,538	1,600	0,894
0,000	0,000	0,000			0,000	0,000	0,000	0,000	0,000
0,003	0,054	0,286	1,000		0,023	0,211	1,429	1,600	0,894
0,776					3,153				
0,379					0,077				

CHROMOSOMAL BREAKS					CHROMATID BREAKS					
All subjects			Subjects with chromosomal br.		All subjects			Subjects with chromatid br.	
Mean	SD	Share of subj. (%)	Mean	SD	Mean	SD	Share of subj. (%)	Mean	SD
21	22	23	24	25	26	27	28	29	30
1,157	0,980	73,100	1,583	0,798	1,838	0,928	97,460	1,885	0,891
0,497	0,660	41,180	1,206	0,446	1,464	0,726	93,460	1,566	0,634
0,871	0,856	59,143	1,469	0,729	1,675	0,860	95,714	1,749	0,806
51,418			12,358		16,801			13,313	
0,000			0,001		0,000			0,000	

in 22.88 % of students (1.05 on average) and in 39.09 % of technicians pending employment (1.49 on average). In one case, the presence of a ring chromosome was found in a technician pendingemployment, while such aberrations were not found in students. The average value for rings is therefore 0.0051 per subject for technicians. In five persons same population, dicentric chromosomes were found. The average value of dicentric chromosomes for this group is 1.6, and 0.0406 for all technicians.

The number of chromosomal breaks in technicians is 1.15 per subject and only 0.49 in students; for chromatid breaks, it is 1.83 per subject and 1.46 in students.

Chromosomal breaks were present in 41.18 % of students (1.20 on average) and in 73.10 % of technicians (1.58 on average); chromatid breaks were present in 93.46 % of students (1.56 on average) and in 97.46 % of technicians (1.88 on average). The differences between the groups of students and technicians pending employment were found to be statistically significant for the percentage CA test, the number of acentric fragments, chromatid and chromosomal breaks. The SCE and MN tests were performed only on a smaller number of test subjects. The average result of the SCE test for students was 6.28 and 6.52 for technicians per 50 cells; for MN test the average results were 4.00 for students and 5.89 per 500 CB cells for technicians. The differences between the two compared groups were not statistically significant (Table 2).

It can be seen from mutagenetic questionary that during the present length of service examined subjects had not worked with sources of ionising radiation. There is a considerable difference in the total duration of service between the two groups, since the technicians have in average up to 7 years of service, while the majority of students have none. The age differences are also significant, since the technicians are on average 11 years older than students.

With the comparison of mutagenetic tests for the two groups we have established that the percentages of CA, the number of acentrics, chromosomal and chromatid breaks increase almost linearly with age. The differences in age groups are statistically significant in % CA, acentrics and chromosomal breaks (Table 4).

While examining the influence of smoking on our results it was established that there were no significant differences in mutagenetic tests between smokers and non-smokers, except for the values for leukocytes (which are higher than average both in technicians and in students who smoke). In these two groups, the SCE test was performed only on a small number of subjects. In their smoking history is very short (smoking history for students is 1.5 years). Student smokers (26 %) smoke on average only 8 cigarettes per day, while for technician smokers (41 %) the average duration of smoking is 6.8 years and they smoke on average 14 cigarettes per day).

Table 2. Mutagenetic testing of subject – SCE and MN test.

Tests		SCE TEST				MN TEST			
No. Groups of test subj.		No. of subjects	No. of exam. cells	Mean – per 50 cells	SD	No. of subjects	No. of exam. cells	Mean – per 500 CB cells	SD
0	1	2	3	4	5	6	7	8	9
1	Technicians	115	5750	6,528	0,714	113,000	56500,000	5,896	2,122
2	Students	5	250	6,268	0,292	5,000	2500,000	4,000	1,581
3	Total	120	6000	6,517	0,702	118,000	59000,000	5,815	2,132
	Variance FR			0,651				3,880	
	analysis FP			0,421				0,051	

Table 3. Length of service and age of test subjects.

Variable		Length of service – at present (years)			Lenth of service – total (years)			Age (years)		
No. Groups of test subj.		No. of subjects	Mean	SD	No. of subjects	Mean	SD	No. of subjects	Mean	SD
0	1	2	3	4	5	6	7	8	9	10
1	Technicians	197	0,000	0,000	197	6,944	8,587	197	32,906	9,395
2	Students	153	0,059	0,367	153	0,059	0,367	153	21,597	1,983
3	Total	350	0,026	0,244	350	3,917	7,279	350	27,934	9,092
	Variance FR		5,066			98,182			214,057	
	analysis FP		0,025			0,000			0,000	

Table 4. Mutagenetic tests according to age of groups of subjects – Structural chromosomal aberrations.

No.	Age groups of subjects (years)	No. of subjects	No. of exam. cells	% CA All subjects Mean	SD	ACENTRICS All subjects Mean	SD	Subjects with acentrics Share of subj. (%)	Mean	SD
0	1	2	3	4	5	6	7	8	9	10
1	Till 18	5	1000	1,000	0,354	0,200	0,447	20,000	1,000	0,000
2	19–25	183	36600	1,195	0,666	0,236	0,487	21,311	1,103	0,384
3	26–35	102	20400	1,639	0,693	0,446	0,640	36,274	1,216	0,417
4	36–45	39	7800	2,218	1,455	0,923	1,345	51,282	1,800	1,399
5	46–55	13	2600	2,269	1,666	1,308	2,394	61,538	2,125	2,800
6	56–65	8	1600	2,438	0,729	1,250	0,707	87,500	1,429	0,535
7	Total	350	70000	1,504	0,849	0,437	0,812	32,000	1,357	1,030
	Variance analysis FR			15,255		9,695			2,421	
	FP			0,000		0,000			0,040	

RINGS All subjects Mean	SD	Subjects with rings Share of subj. (%)	Mean	SD	DICENTRIC CHROMOSOMES All subjects Mean	SD	Subjects with dicentric chrom. Share of subj. (%)	Mean	SD
11	12	13	14	15	16	17	18	19	20
0,000	0,000	0,000	0,000		0,000	0,000	0,000	0,000	0,000
0,000	0,000	0,000	0,000		0,011	0,105	1,093	1,000	0,000
0,000	0,000	0,000	0,000		0,000	0,000	0,000	0,000	0,000
0,000	0,000	0,000	0,000		0,128	0,570	12,821	2,500	0,707
0,077	0,277	7,690	1,000		0,077	0,277	7,692	1,000	0,000
0,000	0,000	0,000	0,000		0,000	0,000	0,000	0,000	0,000
0,003	0,054	0,286	1,000		0,023	0,211	2,286	1,600	0,500
5,487					2,492			5,400	
0,000					0,031			0,156	

CHROMOSOMAL BREAKS All subjects Mean	SD	Subjects with chromosomal br. Share of subj. (%)	Mean	SD	CHROMATID BREAKS All subjects Mean	SD	Subjects with chromatid br. Share of subj. (%)	Mean	SD
21	22	23	24	25	26	27	28	29	30
0,600	0,548	60,000	1,000	0,000	1,200	0,447	100,000	1,200	0,447
0,571	0,745	44,262	1,300	0,560	1,571	0,803	94,536	1,663	0,727
1,099	0,878	72,549	1,500	0,667	1,733	0,747	97,059	1,786	0,692
1,436	1,071	87,179	1,647	0,981	1,949	1,297	97,436	2,000	1,273
1,231	0,927	76,923	1,600	0,699	1,846	0,801	100,000	1,846	0,801
1,625	1,598	62,500	2,600	1,140	2,000	1,069	87,500	2,286	0,756
0,871	0,856	59,143	1,469	0,729	1,675	0,860	95,714	1,749	0,806
11,091			4,322		2,047			2,333	
0,000			0,001		0,072			0,042	

Discussion

The determination of the frequency of chromosomal aberrations in peripheral blood lymphocytes as a biological indicator of the effect of genotoxic agents has been used in practice for a long period of time. During this time, a large number of papers were published which presented data on the frequency of spontaneous aberrations, especially those of the chromosomal type. With regard to the fact that the spontaneous incidence of genome damage is essentially influenced by individual non-identified agents and regional, ecologically specific features, we are interested in comparing the recent situation with values from 10 or more years ago. The most systematic analysis of this type was performed by Lloyd in 1984. Even though he focused only on the spontaneous occurrence of dicentric and acentric fragments, he also analysed other available data on chromatid and chromosomal lesions.[10] He analysed

Table 5. Mutagenetic testing of subjects – SCE and MN tests.

No. Age groups of subj. (years)	Tests	No. of subjects	No. of exam. cells	SCE TEST Mean – per 50 cells	SD	No. of subjects	No. of exam. cells	MN TEST Mean – per 500 CB cells	SD
0	1	2	3	4	5	6	7	8	9
1	19–25	23	1150	6,228	0,658	23	11500	4,913	2,151
2	26–35	62	3100	6,466	0,551	61	30500	5,817	1,873
3	36–45	23	1150	6,737	1,021	22	11000	6,509	2,516
4	46–55	7	350	6,763	0,434	7	3500	6,286	1,976
5	56–65	5	250	7,096	0,664	5	2500	6,600	2.702
6	Total	·120	6000	6,517	0,702	118	59000	5,815	2,132
Variance	FR			2,816				1,921	
analysis	FP			0,029				0,112	

Table 6. Length of service and age of test subjects according to age groups.

No. Age groups of subj. (years)	Variable	Length of service – at present (years) No. of subjects	No. of exam. cells	SD	Length of service – total (years) No. of subjects	No. of exam. cells	SD	Age (years) No. of subjects	No. of exam. cells	SD
0	1	2	3	4	5	6	7	8	9	10
1	do 18	5	0.000	0,000	5	0,000	0,000	5	18,367	0,070
2	19–25	183	0.044	0,326	183	0,412	1,142	183	21,885	1,760
3	26–35	102	0.010	0,100	102	4,525	4,711	102	29,390	2,635
4	36–45	39	0.000	0,000	39	11,811	8,366	39	39,537	2,720
5	46–55	13	0.000	0,000	13	16,615	14,245	13	49,730	3,033
6	56–65	8	0.000	0,000	8	22,000	15,062	8	59,913	3,168
7	Total	350	0.026	0,244	350	3,917	7,279	350	27,934	9,092
Variance	FR		0.427			70,792			1074,692	
analysis	FP		0.830			0,000			0,000	

a total of 65 different mutagenetic studies with over 2000 test subjects, i.e. 211,660 examined cells. The values for dicentric fragments are mainly in the range from 1 to (more rarely) 2/1000. Since his studies included test subjects from a wide age interval, our data differ somewhat from his. Not one dicentric fragment or ring chromosome was found in our group of students, while in the group of technicians (which was composed of subjects of greater age differences and longer smoking periods, as well as the influence of other lifestyle factors), dicentric and centric ring chromosomes were found in 0.011 %. Acentric fragments were found in both groups in 0.217 %. Lloyd stated that in the majority of publications he analysed, the value for acentric fragments was about 3 x 10-³. In a study which included 304 subjects, Galloway et al. (1986) found a frequency of dicentrics of 2.1 x 10-³ and 3.2 x 10-³ for acentric fragments.[11] Bender et al. (1988) found 1.6 x 10-³ dicentrics and 4.6 x 10-³ acentric fragments in a mixed black-white American population.[7] In addition to individual standard aberrations, Awa and Neel (1986) also state data on the pres-

ence of a certain number of "rogue" cells with multiple dicentric, tricentric and acentric fragments and breaks. No explanation for this phenomenon is given.[12] Such types of changes were not found in this study, even though they were present in certain other of our studies.

The comparative analysis of the frequency of chromatid aberrations revealed certain problems. This type of aberrations involves changes which usually do not originate from the circulating Go population of lymphocytes, but are formed either during or after the phase of DNA synthesis, i.e. *in vitro*.

There are also differences regarding the classification of chromosomal changes: true chromatid breaks with larger or smaller dislocations of the broken fragment of one chromatid, and gaps – achromatic regions on chromatids. The latter are not a true damage to the genome, but most often merely a change in the condensation of the protein part of chromosomes, while DNA continuity is preserved.

Galloway found 0.64 % of chromatid deletions in the range of 0 to 6 and chromatid exchanges in 0.11 %.[11] In 7000 examined metaphases of the con-

trol population, Karačić et al. (1995) found 0.48 % chromatid breaks, 0.27 % isochromatid chromosome breaks and 0.23 % acentric fragments. Dicentric and centric rings were not found.[13]

Our data with 0.837 % chromatid breaks per subject not including gaps is comparable to the data of other authors. At the same time, these values can serve as a good indicator of the conditions of in vitro cultivation. This type of damage differs for different authors, since it can be caused by the conditions of in vitro cultivation, including the quality of culture medium, serum, temperature, centrifugation, etc. Isochromatid-chromosomal breaks, whose presence in the first in vitro division indicates G1 damage, was found in 0.248 % of the student group and in 0.5787 % of the technician group. The difference between the two groups is significant (p= 0.00) and may indicate the influence of age. It does not only include physiological differences caused by age, but especially lifestyle factors, which are expressed at older age.

With all the simplicity and quickness of the technical procedure, as well as the possibility of machine (automatic) processing, the micronucleus test as the universal indicator of exposure to genotoxic substances shows a large variability in the number of micronuclei per 500 or 1000 analysed cells prepared according to the same protocol.[14] Certain authors found 20 or more micronuclei per 1000 CB lymphocytes, while others state 3 to 5 per thousand.[5,9,15] This non-uniformity of data and the resulting non-availability of universal reference values dictate the need for collecting one's own data for the general population.

Our results per 59000 CB cells indicate the frequency of micronuclei of 5.815 ± 2.132 (CB cells). This data is therefore the background value for the professionally non-exposed Slovene population.

The reference value for the SCE test which is nowadays regularly used in all population mutagenetic studies is 6.52 ± 0.702 per metaphase for professionally non-exposed Slovenian population, which is similar to data stated by numerous authors.[5,16,17] No significant differences in the incidence of SCE per metaphase with regard to age were noticed. This test which can be considered as a mutagenetic method of choice for the detection of exposure to chemical genotoxic agents showed very small individual deviations in our study. For this reason it is considered to be a reliable indicator for the assessment of combined exposure to chemical and physical agents.

The stated values of mutagenetic tests carried out on 350 test subjects provide a good orientation value for mutagenetic monitoring and large ecological studies, or for the analysis of specific groups professionally exposed to genotoxic agents.

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