

The effects of biological and life-style factors on baseline frequencies of chromosome aberrations in human lymphocytes

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Key words: Age, biomarkers of cancer

risk, chromosomal aberrations, human

lymphocytes, sex

ABSTRACT

Objective: This study investigated the influence of sex and ageing on chromosomal damage and the role of life-style habits on the frequency of chromosomal aberrations (CAs) in peripheral blood lymphocytes (PBLs) of healthy Bosnian subjects. Materials and Methods: Peripheral blood samples were obtained from 100 healthy, unrelated individuals in Bosnia and Herzegovina during 2010 and 2011. Chromosome preparations were made by dropping and air drying and slides were stained with 10% Giemsa solution (pH 6.8). The cytogenetic analysis was carried out in a cytogenetic laboratory in the Department of Biology of the Faculty of Science in Sarajevo. The category of total structural CAs was sub classified as chromosome-type aberrations (CSAs) and chromatid-type aberrations (CTAs) while the category of total numerical CAs was sub classified as aneuploid and polyploid mitoses. All statistical analyses were carried out using Microsoft Excel 2010 (Microsoft Corporation) and the Windows Kwikstat Winks SDA 7.0.2 statistical software package (Texa Soft Cedar Hill, Texas). Results: Cytogenetic analysis revealed the average number of structural CAs was 2.84 and of numerical CAs was 9.56. There was a significant increase in the frequency of chromosome-type aberrations (1.92) compared with chromatid-type aberrations (CTAs) (0.92) and a significant increase in the frequency of an uploid (8.83) compared with polyploid (0.73) mitoses. Significant positive correlations between age and CTAs in human PBLs were also demonstrated. Additional statistical analysis showed that ageing increase number of numerical CAs in lymphocytes of drinkers. The frequency of structural CAs of females exposed to radiation was significantly greater than in males. Analysis indicates the presence of a positive association between CAs and smoking in younger subjects but a negative correlation between aberrant cells frequencies and alcohol in older drinkers. **Conclusion:** The results of the study support the conclusion that sex and age, together with life-style habits of individuals as confounding factors can affect spontaneous frequency of CAs especially to CTAs in PBLs that are a biomarker of cancer risk.

Access this article online					
Quick Response Code:	Website: www.archivepp.com				
	DOI: 10.4103/2045-080X.128372				

INTRODUCTION

Mutation refers to any heritable changes in the genetic material in cells brought about spontaneously or induced by exposure to chemical or physical mutagens. Spontaneous levels of chromosomal aberrations (CAs) are influenced by many exogenous and endogenous factors such as age, viral infections, medical treatments (chemotherapy and radiation), the presence of genotoxicants in food and in the environment (air, drinking water), immunological, hormonal and repair conditions and genetic susceptibility. Therefore, in different parts of the world different spontaneous levels of CAs are found.^[1]

Because both CAs and gene mutations are known to be involved in inherited disorders as well as in the etiology of human tumors, it is important to develop methods to assess these events after human exposure to genotoxic agents. The most common tests used are those showing chromosome aberrations. In general, this test is performed on peripheral blood lymphocytes (PBLs) in subjects occupationally exposed to agents such as ionizing radiation (radiation dosimetry) or chemical mutagens and environmental.

The frequency of CAs in PBLs of healthy individuals represents a cytogenetic marker of the biological effects of mutagen and carcinogen exposure. The variation in the frequency of CAs at the population level remains poorly understood, particularly with respect to the diversity of genotype and other factors that modify it. Results from the cohort studies showed that CA frequency was not modified by sex, age, or smoking habits,^[2,3] but demonstrated that sex influences the frequencies of micronuclei, with females showing higher mean levels than males.^[4] Some studies have found a significant influence of age on CA frequency,^[5] whereas others have found no association at all.^[6] Recently, age, together with other life-style factors, was found to be strongly associated with the frequency of CAs.^[7] The results were obtained by Mladinic et al.[8] indicate that the accumulation of genome damage observed in leukocytes of elderly subjects is intertwined with the ageing process.

The aim of this study was to investigate the effects of sex and ageing on spontaneous chromosomal damage and the potential influences of major confounding factors such as a smoking habits, alcohol consumption, family history of cancer, medical treatments including medication (chemotherapy) and exposure to diagnostic X-rays on the background levels of CAs and their type in PBLs of healthy Bosnian subjects. Furthermore, the study was tried to estimate of the association between CA frequency and cancer risk.

MATERIALS AND METHODS

Study population

The study was approved by the Federal Ministry of Education and Science. Peripheral blood samples were obtained from 100 healthy, unrelated individuals in Bosnia and Herzegovina during 2010 and 2011. All participating subjects signed an informed consent form prior to their inclusion in the study and completed a questionnaire on their demographic data and life-style including smoking status and intake of alcohol, disease or cancer history, occupational history and exposure to medication or environmental agents to evaluate the potential confounding influences in the data analysis. Information on cancer incidence for 2010 and 2011 year was collected through linkage with national cancer and cause of death registries^[9,10] and then incidence rates were estimated (number of cases/100,000 persons/year).

Lymphocyte sampling

Heparinized venous blood samples (3 ml) were obtained from each subject and transported to the cytogenetic laboratory in an insulated ice box or samples were obtained in the laboratory. Short-term lymphocyte cultures were established within 24 h of sampling according to the method of Moorhead *et al.*^[11] with minor modifications.

Whole blood cultures for human lymphocytes

The PBLs were incubated at 37°C for 72h; a blood sample of 0.5 ml was inoculated into a flask containing 7 ml of RPMI-1640 medium (Sigma-Aldrich) supplemented with 20% fetal bovine serum and 0.01 ml of 5,000 units penicillin and 5 mg streptomycin/ml (Sigma-Aldrich). 0.2 ml phytohemagglutinin (PHA-P; Sigma-Aldrich) was added to stimulate cell division (10 µg/ml of medium). The first metaphase after the addition of the mitogen is not reached for about 36-40 h, after which the cells divide about every 18 h. To block the mitotic process of the cells at the metaphase stage, Demecolcine (Sigma-Aldrich) was added for the last 2 h of culture at a final concentration of $10 \mu g/ml$. At the end of the growth period, cells were collected by centrifugation, resuspended in a prewarmed hypotonic solution (0.075 M KCl) for 60 min and fixed in acetic acid/methanol (1:3). The treatment with fixative was repeated 4 times. Chromosome preparations were made by dropping and air drying and slides were stained with 10% Giemsa solution (pH 6.8). Slides from each culture were numbered and scored blind.

Cytogenetic analysis

The cytogenetic analysis was carried out in a cytogenetic laboratory in the Department of Biology of the Faculty of Science in Sarajevo. The frequency of CAs was analyzed in lymphocytes cultured for 72 h. The number of aberrations was observed under a Jenaval microscope, using the ×1000 magnification objective. For each person, 100 well-spread metaphases were analyzed. CAs were recorded without karyotyping. A cell was considered to be aberrant if it had at least one unstable CA. Structural and numerical CAs, the number of aberrant metaphases and the total number aberrations were examined. Cytogenetic markers were always evaluated according to standard protocols in PHA-P-stimulated PBLs. The category of total structural CAs was sub classified as chromosome-type aberrations (CSAs) and chromatid-type aberrations (CTAs) while the category of total numerical CAs was sub classified as aneuploid and polyploid mitoses.

Statistical analysis

In order to explore the genotoxicity of unhealthy life-style, we evaluated the effect of some individual life-style factors on CA frequency in cultured human lymphocytes. Data were collected by the questionnaire on variables including personal habits, such as smoking status (the number of cigarettes smoked per day and the number of years smoking), alcohol consumption, family history of cancer and exposure to medication (medical use of drugs) and radiation (dental or other diagnostic X-ray exposures) or environmental genotoxicants. The subjects were divided into groups according to their sex, age and life-styles based on their responses



Figure 1: Metaphase with multiple structural aberrations (chromosome-type aberrations: Acentric fragments, dicentrics and polycentrics, chromosome-type breaks and chromosome minute fragments; chromatid-type aberrations: Chromatid minute fragments and chromatid-type breaks) (×1000)

to a questionnaire regarding habits. Smoking habit was ranked as smoker (individuals who, for at least 2 years, had been consumed four or more cigarettes per day) or non-smoker. Similarly, frequency of alcohol consumption was classified as drinker (individuals who consumed alcoholic beverages once a week at least) or non-drinker. Individuals exposed to medication (one to three medical treatments in the year before samples were collected) or radiation (a diagnostic exposure in the year before samples were collected) and environmental genotoxicants were grouped into exposed or unexposed subjects. Family history of cancer was classified as family with cancer case (one to three family members of the subject had or have different type of cancer through three generations) or no cancer. Furthermore, each group was sub classified regarding to sex (females and males) and age (<40 and \geq 40 years).

Data were expressed as the mean \pm standard deviation of the means. To evaluate the association between age, sex or confounding factors and each cytogenetic markers simple linear regression analyses was performed. Significant differences between the life-style factors and cytogenetic marker levels were estimated by a Student's *t*-test (comparing two means), when differences between two groups were compared. The term "statistically significant" refers to $P \le 0.05$. All statistical analyses were carried out using Microsoft Excel 2010 (Microsoft Corporation) and the Windows Kwikstat Winks SDA 7.0.2 statistical software package (Texa Soft Cedar Hill, Texas).

RESULTS

The study population comprised of 100 participants of which 63 were females and 37 were males. Their average



Figure 2: Aneuploid metaphase with 45 chromosomes – Giemsa staining (×1000)



Figure 3: Polyploid mitosis (×1000)

age was 33.89 ± 14.33 years (range 16-69); the mean ages of females and males were 31.90 ± 14.13 (range 16-63) and 37.27 ± 14.21 years (range 16-69), respectively.

The cytogenetic analyses of metaphase lymphocytes from this population found 1199 aberrant cells to have a total of 1240 structural and numerical CAs. The overall number of structural CAs was 284 (22.9% of total detected aberrations in human lymphocyte cultures, with a mean of 2.84 ± 2.55 structural CAs), of which CSAs represent 67.61% and chromatid-type 32.39%. There was a significant increase (t = 4.19; P < 0.001) in the frequency of CSAs (1.92 ± 2.11) compared with the CTAs (0.92 ± 1.11). The following CSAs were observed: 45.1% acentric fragments, 18.3% dicentrics, 2.1% chromosome-type breaks and 2.1% chromosome minute fragments. The CTAs included chromatid minute fragments (16.5%) and chromatid-type breaks (15.8%). In two samples, cells with more than five aberrations were observed [Figure 1]; these were not included in the statistical analysis. There were 956 numerical CAs (77.1% of the total aberrations), of which aneuploidy [Figure 2] represented 92.4% and polyploidy [Figure 3] only 7.6%, giving a mean of 9.56 ± 8.66 numerical CAs. The overall numerical CA frequency in human PBLs was significantly higher (t = 7.44; P < 0.001) than that of structural CAs. Among the numerical CAs, there were significantly more an euploid (8.83 ± 8.44) than polyploidy (0.73 ± 1.04) mitoses (*t* = 9.53; *P* < 0.001).

The frequency of structural CAs was higher in females (182, with mean 2.89 \pm 2.62) than in males (102, with mean 2.76 \pm 2.45), but numerical CAs occurred more frequently in males (405, with mean 10.95 \pm 11.36) than in females (551, with mean 8.75 \pm 6.55). The frequency of aberrant metaphases in male individuals was greater than in females and the total number of aberrations in male lymphocytes was also increased [Table 1]. When differences between two means of male and female subjects were compared using a *t*-test, differences were not statistically significant for structural CAs, numerical CAs, number of aberrant metaphases, or for structural and numerical aberrations together. The results of the study clearly suggest a significant positive correlation between age and the CTAs (r = 0.2000; P = 0.046).

An increased frequency of numerical CAs, aberrant mitoses and the total number of aberrations in lymphocytes was also found in subjects of 40 years old or more compared to younger subjects, but this was not statistically significant. There was a slight non-significant increase in the frequency of structural CAs in the younger sample set.

A small but not statistically significant increase in numerical CAs, aberrant mitoses and the total number of aberrations was observed in non-smoker, non-drinkers and persons with no cancer in the family, whereas an increase in structural CAs was found in non-smokers. Subjects exposed to medication or radiation had a higher number of cytogenetic biomarkers except for numerical CAs of subjects that have been exposed to radiation, but this was not statistically significant.

After including sex or age for each group of life-style factors, the older age groups had a higher number of numerical CAs, aberrant cells and total aberrations at all categories (sex, age, smoking habits, alcohol consumption, family history of cancer and exposure to medication or environmental genotoxicants) than younger age groups and had more structural aberrations in a group of male, cancer case and smoker than younger subjects. Statistically significant increased frequency of numerical CAs was found in the older age group of drinkers (P = 0.031). In regard with sex, females had increased frequency of structural aberrations in all groups except in a group of smokers and males had increased frequency of numerical CAs, the number of aberrant metaphases and the total number of aberrations in all groups except in the group exposed to medication or radiation. The frequency of structural CAs in female individuals exposed to radiation was significantly greater than in males (P = 0.019).

The Pearson correlations were used to analyze relationships between selected pairs of variables: Age, sex or confounding factors and each cytogenetic

Table 1: Effects of demographic factors on frequency of cytogenetic markers in populations (mean±SD)							
Group	N	Structural aberrations	Numerical aberrations	Aberrant cells	Total aberrations	Mean age (years)±SD	
Total	100	2.84±2.55	9.56±8.66	11.99±9.59	12.40±9.68	33.89±14.33	
Female	63	2.89±2.62	8.75±6.55	11.16±7.04	11.63±7.22	31.90±14.13	
40≥	21	2.62±2.18	9.57±8.33	11.62±8.35	12.19±8.36	50.29±6.75	
40<	42	3.02±2.83	8.33±5.52	10.93±12.79	11.36±6.67	22.71±4.50	
Male	37	2.76±2.45	10.95±11.36	13.41±12.82	13.70±12.86	37.27±14.21	
40≥	17	2.76±2.91	13.65±14.40	15.88±16.54	16.41±16.53	50.76±7.89	
40<	20	2.75±2.07	8.65±7.62	11.30±16.83	11.40±8.43	25.80±5.36	
Cancer case	26	3.00±2.42	9.35±8.22	11.69±8.13	12.35±8.25	35.69±14.75	
Female	21	3.29±2.53	8.19±8.62	10.81±8.61	11.48±8.70	41.90±14.90	
Male	5	1.80±1.48	14.20±3.77	15.40±4.56	16.00±5.10	39.00±15.28	
No cancer	74	2.78±2.61	9.64±8.86	12.09±10.11	12.42±10.19	33.26±14.22	
Female	42	2.69±2.67	9.02±5.32	11.33±6.23	11.71±6.47	30.40±13.67	
Male	32	2.91±2.56	10.44±12.09	13.09±13.69	13.34±13.70	37.00±14.28	
Cancer case	26	3.00±2.42	9.35±8.22	11.69±8.13	12.35±8.25	35.69±14.75	
40≥	13	3.00±2.20	12.15±10.38	14.23±10.08	15.15±9.99	49.62±5.44	
40<	13	3.00±2.71	6.54±4.01	9.15±4.69	9.54±4.99	21.77±1.96	
No cancer	74	2.78±2.61	9.64±8.86	12.09±10.11	12.42±10.19	33.26±14.22	
40≥	25	2.52±2.66	11.00±12.18	13.16±14.01	13.52±14.02	50.96±8.01	
40<	49	2.92±2.60	8.94±6.61	11.55±7.50	11.86±7.66	24.22±5.39	
Smoker	41	2.73±2.44	9.41±10.38	11.68±11.79	12.15±11.89	36.24±14.16	
Female	27	2.59±2.17	7.56±5.29	9.67±5.84	10.15±6.22	35.67±15.13	
Male	14	3.00±2.96	13.00±15.95	15.57±18.30	16.00±18.26	37.36±12.52	
Non-smoker	59	2.92±2.64	9.66±7.32	12.20±7.81	12.58±7.90	32.25±14.34	
Female	36	3.11±2.93	9.64±7.29	12.28±7.71	12.75±7.78	29.08±12.83	
Male	23	2.61±2.15	9.70±7 <mark>.52</mark>	12.09±8.13	12.30±8.25	37.22±15.42	
Smoker	41	2.73±2.44	9.41±10.38	11.68±11.79	12.15±11.89	36.24±14.16	
40≥	18	3.06±2.71	1 <mark>1.67±1</mark> 4.41	14.00±16.49	14.72±16.44	50.44±6.69	
40<	23	2.48±2.23	7.65±5.31	9.87±5.91	10.13±6.22a	25.13±5.99	
Non-smoker	59	2.92±2.64	9.66±7.32	12.20±7.81	12.58±7.90	32.25±14.34	
40≥	20	2.35±2.30	11.15±8.37	13.10±8.30	13.50±8.36	50.55±7.78	
40<	39	3.21±2.78	8.90±6.71	11.74±7.62	12.10±7.72	22.87±4.11	
Drinker	18	3.11±2.61	8.22±5.62	10.83±6.20	11.33±6.32	34.28±14.63	
Female	8	3.13±3.04	6.88±5.41	9.63±7.76	10.00±7.65	28.63±15.24	
Male	10	3.10±2.38	9.30±5.83	11.80±4.83	12.40±5.21	38.80±13.13	
Non-drinker	82	2.78±2.55	9.85±9.19	12.24±10.20	12.63±10.29	33.80±14.35	
Female	55	2.85±2.58	9.02±6.70	11.38±6.98	11.87±7.20	32.38±14.05	
Male	27	2.63±2.51	11.56±12.87	14.00±14.77	13.89±14.59	36.70±14.79	
Drinker	18	3.11±2.61	8.22±5.62	10.83±6.20	11.33±6.32	34.28±14.63	
40≥	7	2.86±2.34	11.71±4.86*	13.57±3.95a	14.57±3.95	50.29±8.32	
40<	11	3.27±2.87	6.00±5.06	9.09±6.88	9.27±6.83	24.09±5.36	
Non-drinker	82	2.78±2.55	9.85±9.19	12.24±10.20	12.63±10.29	33.80±14.35	
40≥	31	2.65±2.56	11.32±12.54	13.52±13.96	13.97±13.95	50.55±7.06	
40<	51	2.86±2.56	8.96±6.35	11.47±7.07	11.82±7.28	23.63±4.93	
Exposed to medication	14	2.93±1.86	12.57±10.03	15.21±9.59	15.50±9.56	46.43±15.20	
Female	7	3.43±1.81	13.29±13.80	16.43±13.00	16.71±12.85	41.29±17.46	
Male	7	2.43±1.90	11.86±5.11	14.00±5.16	14.29±5.41	51.57±11.57	
Unexposed	86	2.83±2.65	9.07±8.38	11.47±9.55	11.90±9.67	31.85±13.18	
Female	56	2.82±2.71	8.18±4.96	10.50±5.79	11.00±6.09	30.73±13.39	
Male	30	2.83±2.59	10.73±12.43	13.27±14.09	13.57±14.1	33.93±12.73	
Exposed to medication	14	2.93±1.86	12.57±10.03	15.21±9.59	15.50±9.56	46.43±15.20	
40≥	10	2.80±2.04	12.80±11.25	15.20±10.87	15.60±10.83	54.20±9.34	
40<	4	3.25±1.50	12.00±7.44	15.25±6.60	15.25±6.60	27.00±5.89	
Unexposed	86	2.83±2.65	9.07±8.38	11.47±9.55	11.90±9.67	31.85±13.18	
40≥	28	2.64±2.67	10.89±11.70	12.93±13.39	13.54±13.40	49.18±5.90	
40<	58	2,91+2,66	8,19+6,11	10.76+7.03	11.07+7.17	23 48+4 87	

Contd...

Nefic and Musanovic: The effects of biological and life-style factors on baseline frequencies

Table 1: contd							
Group	N	Structural aberrations	Numerical aberrations	Aberrant cells	Total aberrations	Mean age (years)±SD	
Exposed to radiation	22	3.14±3.51	9.50±6.16	12.05±6.56	12.64±6.77	35.36±15.88	
Female	14	4.21±3.96*	9.29±6.49	12.79±6.96	13.50±6.91	32.86±15.69	
Male	8	1.25±1.16	9.88±5.96	10.75±5.99	11.13±6.66	39.75±16.27	
Unexposed	78	2.76±2.23	9.58±9.27	11.97±10.32	12.33±10.40	33.47±13.94	
Female	49	2.51±1.99	8.59±6.62	10.69±7.07	11.10±7.29	31.63±13.82	
Male	29	3.17±2.56	11.24±12.52	14.14±14.13	14.33±13.87	36.59±13.83	
Exposed to radiation	22	3.14±3.51	9.50±6.16	12.05±6.56	12.64±6.77	35.36±15.88	
40≥	10	3.00±2.45	10.20±6.12	12.10±5.38	13.20±5.85	51.10±8.62	
40<	12	3.25±4.31	8.92±6.40	12.00±7.64	12.17±7.67	22.25±2.83	
Unexposed	78	2.76±2.23	9.58±9.27	11.97±10.32	12.33±10.40	33.47±13.94	
40≥	28	2.57±2.54	11.82±12.92	14.04±14.47	14.39±14.43	50.29±6.77	
40<	50	2.86±2.05	8.32±6.22	10.82±6.95	11.18±7.17	24.06±5.31	

Student's t test: *P<0.05 and Pearson's correlation: *P<0.05. SD=Standard deviation

Table 2: The frequency of cytogenetic markers in healthy populations by year										
Group	N	CTAs	CSAs	Structural CAs	Aneuploidy	Polyploidy	Numerical CAs	Aberrant cells	Total CAs	Mean age (years)±SD
Total 2010-2011	100	0.92±1.11	1.92±2.11	2.84±2.55	8.83±8.44	0.73±1.04	9.56±8.66	11.99±9.59	12.40±9.68	33.89±14.33
2010	34	0.56±0.66	2.71±2.84*	3.26±3.20	10.94±12.49	0.59±1.10	11.53±12.95	11.99±14.52	14.79±14.48	29.32±10.82
2011	66	1.11±1.24**	1.52±1.47	2.62±2.13	7.74±5.09	0.80±1.01	8.55±5.09	11.99±5.30	11.17±5.64	36.24±15.38*
2010										
Male	13	0.69±0.63	2.54±2.63	3.23±2.98	13.62±17.31	0.85±1.46	14.46±17.97	11.99±20.56	17.69±20.49	34.46±9.68*
Female	21	0.48±0.68	2.81±3.03	3.29±3.41	9.29±8.37	0.43±0.81	9.71±8.61	11.99±9.20	13.00±9.23	26.14±10.45
2011										
Male	24	1.08±1.41	1.42±1.18	2.50±2.15	8.21±4.91	0.83±1.20	9.04±4.79	11.99±4.68	11.54±5.09	38.79±16.13
Female	42	1.12±1.15	1.57±1.63	2.69±2.15	7.48±5.23	0.79±0.90	8.26±5.29	11.99±5.66	10.95±5.99	34.79±14.94
2010										
Male	13	0.69±0.63	2.54±2.63	3.23±2.98	13.62±17.31	0.85±1.46	14.46±17.97	11.99±20.56	17.69±20.49	34.46±9.68
2011	24	1.08±1.41	1.42±1.18	2.50±2.15	8.21±4.91	0.83±1.20	9.04±4.79	11.99±4.68	11.54±5.09	38.79±16.13
2010										
Female	21	0.48±0.68	2.81±3.03	3.29±3.41	9.29±8.37	0.43±0.81	9.71±8.61	11.99±9.20	13.00±9.23	26.14±10.45
2011	42	1.12±1.15**	1.57±1.63	2.69±2.15	7.48±5.23	0.79±0.90	8.26±5.29	11.99±5.66	10.95±5.99	34.79±14.94*
2010										
40≥	7	0.57±0.79	2.14±3.08	2.71±3.68	21.29±22.01	0.86±1.46	22.14±23.31	11.99±26.63	24.86±26.54	47.86±4.98***
40<	27	0.56±0.64	2.85±2.82	3.41±3.13	8.26±7.06	0.52±1.01	8.78±7.03	11.99±8.28	12.19±8.32	24.52±5.15
2011										
40≥	31	1.26±1.29	1.42±1.15	2.68±2.23	7.94±4.55	1.03±1.17	8.97±4.51	11.99±4.52	11.65±4.88	51.10±7.53***
40<	35	0.97±1.20	1.60±1.72	2.57±2.08	7.57±5.59	0.60±0.81	8.17±5.59	11.99±5.96	10.74±6.28	23.09±4.80
2010										
40<	27	0.56±0.64	2.85±2.82*	3.41±3.13	8.26±7.06	0.52±1.01	8.78±7.03	11.99±8.28	12.19±8.32	24.52±5.15
2011										
40<	35	0.97±1.20	1.60±1.72	2.57±2.08	7.57±5.59	0.60±0.81	8.17±5.59	11.99±5.96	10.74±6.28	23.09±4.80
2010										
40≥	7	0.57±0.79	2.14±3.08	2.71±3.68	21.29±22.01	0.86±1.46	22.14±23.31	11.99±26.63	24.86±26.54	47.86±4.98
2011										
40≥	31	1.26±1.29	1.42±1.15	2.68±2.23	7.94±4.55	1.03±1.17	8.97±4.51	11.99±4.52	11.65±4.88	51.10±7.53

Student's *t* test: **P*<0.05, ***P*<0.01, ****P*<0.001. CTAs=Chromatid-type aberrations, CSAs=Chromosome-type aberrations, CAs=Chromosomal aberrations, SD=Standard deviation

markers in population. Statistical analysis indicates the presence of a positive association between the total number of CAs and smoking in the younger group of smokers (r = 0.4598; P = 0.027). There was statistically significant a negative correlation between the total number of aberrant cells and alcohol in the older age groups of drinkers (r = -0.7559; P = 0.049).

Population estimation of the Federation of Bosnia and Herzegovina was 2,337,660 for year 2010 and 2,338,270 for year 2011. Women make up to 51% whereas men 49%. 68% is aged 15-65 (42, 43). In the year 2010, there were 4123 cancer cases and 4228 in 2011. Crude cancer rate was 176.37/100,000 in 2010 and it was higher in 2011, 180.82/100,000.

Statistically significant (t = -2.89; P = 0.005) increased frequency of CTAs was found in year 2011 (1.11 ± 1.24) than in year 2010 (0.56 ± 0.66) [Table 2]. Similarly, females in year 2011 had statistically significant (t = -2.77; P = 0.008) higher number of CTAs (1.12 ± 1.15) compared with females in 2010 (0.48 ± 0.68). However, the number of CSAs (t = 2.29; P = 0.027) was greater in 2010 (2.71 ± 2.84) than in year 2011 (1.52 ± 1.47). In the year 2010, the subjects younger than 40 years had significantly (t = 2.03; P = 0.049) higher number of CSAs (2.85 ± 2.82) compared with subjects the same age in year 2011 (1.60 ± 1.72).

DISCUSSION

The CAs in human peripheral lymphocytes are biomarkers of exposure to occupational or environmental genotoxic agents. Cytogenetic analyses of lymphocytes from populations exposed to such agents have shown increased CA frequencies. In this study, total numerical CA frequency in human PBLs was significantly higher than structural CAs. There was a significant increase in the frequency of CSAs compared to the CTAs and a significant increase in the frequency of aneuploid mitoses compared to polyploid mitoses. Genetic instability, which includes both numerical and structural chromosomal abnormalities, is a hallmark of cancer.^[12] Structural aberration or rearrangements can have an impact on tumor development through the activation of oncogenes and inactivation of tumor suppressor genes. The role of numerical aberrations during tumor development is less well-understood.

The dicentric chromosomes in the population are indicating a previous exposure to radiation. Few studies of cancer incidence among veterans or peacekeepers serving in the Balkan area or local populations showed a significant increase of chromosome aberrations and cancer cases compared to the control a subjects^[13,14] which some attributed to exposure to depleted uranium. These facts indicating the influence of chromosome aberrations on eventual increase in the number of malignant diseases in Bosnia and Herzegovina.^[15] Whole-chromosome aneuploidy, including hyperdiploidy and hypodiploidy is the somatic mutation that can predispose to tumor development. The aneuploidy hypothesis predicts a carcinogen (or mutant gene) generates aneuploidy that destabilizes the karyotype and thus initiates an autocatalytic karyotype evolution generating preneoplastic and eventually neoplastic karyotypes.^[16]

Previous studies in healthy populations have reported that both known and unknown factors impede the establishment of a universal baseline of CAs. In general, the evidence from the literature on the role of age as a confounding factor in cytogenetic biomonitoring studies is inconclusive. Some studies found a significant effect on CA frequency,^[5] whereas others found no association.^[6] The results of the current investigation clearly suggest a significant positive correlation between age and frequency of CTAs in human PBLs. An age-related increase in aneuploid cells in human lymphocytes has been reported in other studies.^[17] These findings together imply that cytogenetic aberrations could be considered as potential biomarkers of ageing, especially as detection of chromosome aberrations in old people is a relatively simple method for verification of genetic instability.^[18] Ageing and genomic instability could be considered interactive phenomena associated with the accumulation of deoxyribonucleic acid damage in the genome.[19]

These data show significant differences in frequency of structural CAs between females and males exposed to radiation. The number of numerical CAs was higher in the drinker than in non-drinker for older subjects. There was statistically significant correlation between the total number of CAs and smoking habits in the group of younger smoker and negative correlation between the total number of aberrant cells and consumption alcohol in the group of older drinker. These results contrast with some of those of the literature. For example, Andersson^[1] reported no significant effects on CA frequency of sex, age or various life-style factors. A few studies have found no change in sister chromatid exchanges (SCE) or CA incidence with smoking,^[20] whereas in others, an increase in the incidence of SCE and CA in the lymphocytes of smokers,^[21] or a synergistic effect of smoking with exposure to other chemicals,^[22] has been described. Drinking has not been found to have an effect on SCE, except at the level consumed by alcoholics, when an increase in both CA and SCE has been observed.^[23] Hospital workers exposed to low-level radiation had an increase in CA frequency compared with workers from the same hospital who had not been exposed.^[24]

Statistically significant increased frequency of CTAs in 2011 compared to 2010 was strongly associated

with increased frequency of cancer rate. This result supports the conclusion that CTA frequency in PBLs could be a biomarker of cancer risk.

CONCLUSION

This study investigated the effects of sex and ageing on chromosomal damage and the role of life-style habits on the frequency of CAs in PBLs of healthy Bosnian subjects. The average number of structural CAs was 2.84 ± 2.55 and of numerical CAs was 9.56 ± 8.66 . The total numerical CA frequency in human lymphocytes was significantly higher than structural CA frequency. There was a significant increase in the frequency of CSAs compared with CTAs and a significant increase in the frequency of aneuploid compared to polyploidy mitoses.

In addition, significant positive correlations between age and frequency of numerical CAs and between age and CTAs in human PBLs, were also demonstrated. In contrast, the significant increase in numerical and CTA frequencies in lymphocytes with age, increase in structural CA frequency in females exposed to radiation, increase in numerical CA and aberrant cell frequencies in drinkers and increase in the total number of CAs in smokers indicate that sex, age and life-style habits of individuals as confounding factors can affect CA frequency and should be considered during realization of human cytogenetic studies. The results support the conclusion that CTA frequency in PBLs could be a biomarker of cancer risk.

ACKNOWLEDGMENTS

The author would like to thank Federal Ministry of Education and Science of Bosnia and Herzegovina for their support for this work.

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How to cite this article: Nefic H, Musanovic J. The effects of biological and life-style factors on baseline frequencies of chromosome aberrations in human lymphocytes. Arch Pharma Pract 2014;5:19-27.

Source of Support: This work was supported by the Federal Ministry of Education and Science of Bosnia and Herzegovina (03-39-5980-120-1/08). **Conflict of Interest:** None declared.



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