

Variability in Chromosome Aberrations, Sister Chromatid Exchanges, and Mitogen-Induced Blastogenesis in Peripheral Lymphocytes from Control Individuals

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Confidence in results from monitoring genetic end points in environmentally or occupationally exposed individuals can be improved with knowledge of the normal variability of changes in genetic end points in the general population. Confounding effects can be determined, and study interpretation can be improved by correlation of this variability with various lifestyle factors such as sex and age, smoking and drinking habits, viral infections, exposure to diagnostic X-rays, etc. Eight blood samples were taken from each of 24 male and 24 female volunteers over a period of 2 years. Questionnaires pertaining to lifestyle were completed at the time of each sampling. Whole blood was cultured and slides prepared for chromosome aberration (CA) or sister chromatid exchange (SCE) analysis. Separated mononuclear cells were cultured with a range of phytohemagglutinin concentrations, and the maximum level of mitogen-induced blastogenesis was determined by measurement of [³H]thymidine uptake. There was a significant effect of both year and season of sampling for all three end points. Because there was no consistent pattern in 2 successive years, effects were thought to be independent of season. No significant effects in any of the three end points were found with respect to sex or age nor any of the other lifestyle factors, although SCE frequency and mitogen-induced blastogenesis were nearly always higher in females than in males. These results point to the need for concurrent sampling of controls with exposed populations.

Introduction

Assays in human lymphocytes in which cytogenetic end points such as chromosome aberrations (CA) or sister chromatid exchanges (SCE) are evaluated can be used to measure environmental or occupational exposure. More confidence could be placed in measurements of positive effects if there was better knowledge of the normal variability in such cytogenetic end points in the general population. The confounding effect of lifestyle factors could be determined by dividing the population into subgroups. Exogenous lifestyle factors include viral illness, X-ray exposure, smoking, and drinking, and endogenous factors include age and sex. It is therefore useful to investigate cytogenetic end points in the lymphocytes of individuals. In addition, the reactivity of lymphocytes to mitogenic

stimulus (mitogen-induced blastogenesis) indicates the competence of the individual's immune system.

Our previous study found an increased incidence of SCE in females compared to males (1,2) which could not be explained by the additional chromatin found in the second X-chromosome, nor by use of the contraceptive pill. An increased incidence of CA and a reduction in mitogen-induced blastogenesis in females was also found (3). Other workers have found similar results for both CA and SCE (4) and for SCE (5-7); contrasting results have been found for mitogen-induced blastogenesis (8).

Previously, no significant differences were seen in CA frequency with respect to age, smoking, drinking, X-ray exposure, and contraceptive pill use (3). SCE frequency was associated with increasing age (although this was thought to be due to a higher proportion of older females in the study population) and with smoking (1), and mitogen-induced blastogenesis was reduced in males recently exposed to X-rays (3).

Other studies have variously confirmed or contradicted these results. The effect of age on cytogenetic parameters has been measured in control individuals in many studies.

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Some studies found a significant effect on CA frequency (9), where as others found no association (10). Those studies that found an age-related increase in SCE frequency usually reported that this increase disappeared if cigarette smoking was taken into account (11). Mitogen-induced blastogenesis has been reported to decrease with increasing age (12,13).

An increase in the incidence of SCE and CA in the lymphocytes of smokers (14) has been found in many studies, and others have found a synergistic effect of smoking with exposure to other chemicals (15). A few studies have found no change in SCE or CA incidence in control individuals with smoking (16).

Drinking has not been found to have an effect on SCE (17), except at the level consumed by alcoholics, when an increase in both CA and SCE has been observed (18).

Hospital workers exposed to low-level radiation had an increase in CA frequency compared to workers from the same hospital who had not been exposed (19). Balakrishna et al. (20), found that oral contraceptive use affected SCE frequency.

Additional lifestyle factors (including viral illness and caffeine intake) have been taken into account in the present study to augment the database. As pointed out by Carrano and Natarajan (21), not all the factors that affect cytogenetic parameters have been identified.

Viral illness was reported to be associated with an increased incidence of chromosome gaps (10), although only one individual showed an unusually high level of gaps. Colds had no effect in workers exposed to vinyl chloride (22). In a study of a large human population, Bender et al. (11) found that race had no influence on SCE frequency. Caffeine intake was associated with increased SCE frequency in a study of 52 Korean women (23), although caffeine consumption was very low compared to consumption in the UK.

In our previous study, females were sampled immediately after males, and it is possible that this small temporal change accounted for the differences observed between the sexes. The present study is concerned with the variation in parameters measured in a selected population of individuals on different occasions. Again, the lifestyle factors can be taken into account in the data analyses, but in addition, variation introduced by sampling at different times of the year could be measured. Two papers which discussed the effect of season in which the blood samples were taken found no effect on the incidence of cytogenetic "rogue" cells exhibiting an extreme degree of damage (24) and supernumerary chromosomal elements (25). However, Tucker et al. (26) did find a difference in SCE between sampling times, and Littlefield and Goh (27) found a difference in CA between sampling times.

Materials and Methods

Blood Samples

Twenty-four male and twenty-four female volunteers who were members of an amateur sports and social club were studied. Eight samples were collected from each

volunteer at three monthly intervals over a 2-year period beginning in January 1987 (with the exception of two, who left the club during this time). We collected 20 mL blood by venipuncture during the early evening, mixed it with 53 U/mL lithium heparin (Sigma, Poole, UK), and stored the samples at room temperature overnight.

Questionnaires were completed by each volunteer when the first blood sample was taken. These were designed to obtain information about age, sex, occupation, current health status, recent infectious illness, recent immunization, X-ray exposure, diagnostic tests undergone, current medication, past health history, allergy, smoking, drinking, caffeine intake, contraceptive pill use, and pregnancy. At each subsequent blood-sampling time, a shorter, follow-up questionnaire was completed, in which any changes in these factors could be detailed (Tables 1, 2, and 3).

Table 1. Age distribution in the population at start of study (sampling time 1).

Age, years	No. of males	No. of females	Total
18-22	5	7	12
23-27	7	2	9
28-32	2	3	5
33-37	0	3	3
38-42	5	1	6
43-47	1	1	2
48-52	2	5	7
53-57	1	2	3
58-62	1	0	1
Total	24	24	48

Table 2. Smoking, drinking, caffeine intake, allergy, serious illness, and contraceptive use in the population at start of study.

Lifestyle factor	Category	Male	Female	Total
Smoking ^a	Current smoker	5	6	11
	Ex-smoker	8	5	13
	Nonsmoker	11	13	24
Drinking ^b	Low	0	8	8
	Medium	9	13	22
	High	15	3	18
Caffeine intake ^c	Low	0	1	1
	Medium	23	20	43
	High	1	3	4
Allergic condition ^d	Yes	7	8	15
	No	17	16	33
Serious illness ^e	Yes	1	3	4
	No	23	21	44
Contraceptive pill ^f	Current user	—	8	—
	Ex-user	—	12	—
	Nonuser	—	4	—

^aCurrent smoker, >1 cigarette, cigar or pipe per day; ex-smoker, gave up smoking >3 months ago; nonsmoker, never smoked.

^bLow, <2 units per week; medium, 2-20 units per week; high, >20 units per week; 1 unit = 1/2 pint beer or 1 glass wine, or 1 measure liquor.

^cLow, <2 cups per day; medium, 2-8 cups per day; high >8 cups per day; 1 cup = 1 cup of coffee, tea, or cola.

^dHay fever, asthma, or any other allergic condition.

^eTuberculosis, hepatitis, or other serious or unusual illness.

^fCurrent user, currently taking contraceptive pill; ex-user, stopped taking contraceptive pill >3 months ago; nonuser, never took contraceptive pill.

Table 3. X-ray exposure, infectious illness, immunization, medication, and pregnancy in the population at each sampling time.

Lifestyle factor	Sex	Year 1, 1987				Year 2, 1988				Total
		W	Sp	Su	A	W	Sp	Su	Au	
X-ray exposure ^a	M	11/24	1/24	4/23	6/23	1/23	4/23	1/23	8/23	31/186
	F	8/24	5/24	3/24	2/24	1/24	3/23	2/23	0/22	24/188
	Total	19/48	6/48	7/47	8/47	2/47	7/46	3/46	8/45	55/374
Infectious illness ^b	M	6/24	7/24	2/23	4/23	6/23	1/23	3/23	3/23	32/186
	F	9/24	6/24	4/24	6/24	8/24	4/23	2/23	4/22	43/188
	Total	15/48	13/48	6/47	10/47	14/47	5/46	5/46	7/45	75/374
Immunization ^c	M	0/24	0/24	0/23	1/23	0/23	1/23	0/23	0/23	2/186
	F	0/24	0/24	0/24	1/24	0/24	0/23	1/23	0/22	2/188
	Total	0/48	0/48	0/47	2/47	0/47	1/46	1/46	0/45	4/374
Medication ^d	M	1/24	1/24	1/23	2/23	1/23	1/23	1/23	1/23	9/186
	F	2/24	4/24	6/24	4/24	3/24	2/23	2/23	6/22	29/188
	Total	3/48	5/48	7/47	6/47	4/47	3/46	3/46	7/45	38/374
Pregnancy ^e	F	1/24	1/24	0/24	0/24	2/24	3/23	4/23	2/22	13/188

Abbreviations: W, winter; Sp, spring; Su, summer; A, autumn.

^aNumber of people out of the total population who have received >1 diagnostic X-ray to limb, teeth, chest, bowel, or other part of the body within the previous 3 months.

^bNumber of people out of the total population who have had glandular fever, measles, flu, chicken pox, mumps, common cold, or other infectious illness within the previous 3 months.

^cNumber of people out of the total population who have been immunized against any illness within the previous 2 weeks.

^dNumber of people out of the total population who were receiving medication or steroids at the time of blood sampling.

^eNumber of people out of the total population who were pregnant at the time of blood sampling; the overall total shows the total number of blood samples taken from pregnant women.

Lymphocyte Cultures

For CA and SCE analyses, heparinized whole blood was used. For mitogen-induced blastogenesis, mononuclear cells were separated and used. The methods of preparing and scoring slides and measuring tritiated thymidine incorporation have previously been described (28).

Statistical Analysis

Initially, correlations were calculated at each of the eight sampling times (for convenience referred to as seasons), between all the lifestyle factors, all the end points (different categories of CA, mean SCE, and mitogen-induced blastogenesis measured as mean maximum phythenagglutinin [PHA] response) and between lifestyle factors and end points.

Analysis of variance was carried out for all end points taking into account variation due to individual, sex, year, and season (and also replicate for SCE) for each of the eight sampling times individually and for the whole study overall. Arcsine transformation was performed on CA data, expressed as the proportion of aberrations before the analysis.

Results

There were no consistent, significant correlations between any of the lifestyle factors and end points examined. Therefore, year, season, and sex were the factors selected to be analyzed in detail.

The results of the evaluation of chromosome aberrations are shown in Table 4. There was no significant difference

($p > 0.05$) for any of the six categories of aberration (gaps, chromatid deletions, chromatid exchanges, chromosome deletions, chromosome exchanges, and "other") or any of the four totals (aberrations per 100 metaphases including gaps, aberrations per 100 metaphases excluding gaps, aberrant metaphases per 100 including gaps, aberrant metaphases per 100 excluding gaps) between males and females at any of the eight sampling times.

Taking the eight sampling times overall, there was a statistically significant effect of the year of sampling for each of the six categories of aberration and each of the four totals (significance levels are shown in Table 5). Mean aberration frequencies from samples obtained in 1987 were always lower than those obtained in 1988. There was a significant effect of the time of sampling for each of the six categories of aberration and the two totals excluding gaps (significance levels are shown in Table 5). Mean aberration frequencies obtained in autumn and winter were nearly always lower than those obtained in spring and summer.

There was a statistically significant interaction between year and season for each category of aberration and totals (significance levels are shown in Table 4B). This was due to the fact that the differences between corresponding seasons in the 2 years were similar in spring, summer, and autumn, but there was no difference between winters. There was no significant effect ($p > 0.05$) of sex and no significant interaction ($p > 0.05$) between year and sex or between year, season, and sex.

The results of the evaluation of SCE are shown in Table 6. There was no significant difference ($p > 0.05$) between males and females at any sampling time. Generally, however, even though effects were not significant, the mean value in SCE tended to be higher in females than in males.

Table 4. Analysis of chromosome aberrations for different seasons of the year.

Aberration	Sex	Mean chromosome aberrations per 100 metaphases								Total	
		Year 1, 1987				Year 2, 1988					
		W	Sp	Su	A	W	Sp	Su	Au		
Gap	M	0.71	0.92	0.18	0.39	0.71	0.35	0.00	0.47	0.48	
	F	0.92	0.97	0.21	0.46	0.75	0.08	0.27	0.30	0.51	
	Total	0.81	0.95	0.20	0.43	0.73	0.22	0.15	0.39	0.50	
Chromatid	Deletion	M	0.38	0.30	0.17	0.13	0.51	0.22	0.16	0.23	0.27
		F	0.50	0.19	0.17	0.33	0.21	0.05	0.45	0.30	0.28
		Total	0.44	0.24	0.17	0.23	0.35	0.14	0.32	0.27	0.27
	Exchanges	M	0.00	0.00	0.00	0.04	0.00	0.00	0.09	0.00	0.02
		F	0.04	0.10	0.00	0.04	0.04	0.00	0.00	0.00	0.03
		Total	0.02	0.05	0.00	0.04	0.02	0.00	0.04	0.00	0.02
Chromosome	Deletions	M	0.17	0.40	0.33	0.13	0.44	0.67	0.86	0.41	0.41
		F	0.38	0.39	0.10	0.33	0.17	0.16	0.08	0.36	0.25
		Total	0.27	0.39	0.22	0.23	0.30	0.42	0.43	0.39	0.33
	Exchanges	M	0.00	0.08	0.04	0.09	0.00	0.00	0.00	0.00	0.03
		F	0.04	0.08	0.06	0.08	0.08	0.00	0.00	0.00	0.05
		Total	0.02	0.08	0.05	0.09	0.04	0.00	0.00	0.00	0.04
Other	M	0.00	0.13	0.14	0.00	0.04	0.00	0.00	0.00	0.04	
	F	0.04	0.04	0.10	0.00	0.08	0.26	0.05	0.05	0.07	
	Total	0.02	0.08	0.12	0.00	0.06	0.13	0.03	0.02	0.06	
Total mean aberrations/100 metaphases	Including gaps	M	1.25	1.72	0.83	0.78	1.71	1.23	1.12	1.12	1.23
		F	1.72	1.67	0.64	1.00	1.25	0.56	0.85	1.01	1.10
		Total	1.48	1.69	0.73	0.89	1.47	0.91	0.97	1.06	1.16
	Excluding gaps	M	0.54	0.86	0.64	0.39	0.99	0.88	1.12	0.65	0.75
		F	0.92	0.75	0.39	0.54	0.58	0.47	0.57	0.71	0.62
		Total	0.73	0.81	0.52	0.47	0.78	0.68	0.82	0.68	0.68
% Aberrant metaphases	Including gaps	M	1.21	1.78	0.87	0.78	1.67	0.98	1.12	1.12	1.20
		F	1.92	1.77	0.64	1.25	1.13	0.56	0.85	0.96	1.15
		Total	1.57	1.77	0.75	1.02	1.39	0.78	0.97	1.04	1.18
	Excluding gaps	M	0.54	0.86	0.69	0.39	0.95	0.80	1.12	0.65	0.74
		F	1.00	0.85	0.39	0.79	0.50	0.47	0.57	0.71	0.67
		Total	0.77	0.85	0.54	0.60	0.72	0.64	0.82	0.68	0.70

Abbreviations: W, winter; Sp, spring; Su, summer; A, autumn.

Table 5. Significance levels measured in the overall analysis of variance for chromosome aberrations for different seasons of the year.

Aberration	Sex	Significance level of variations due to		
		Year	Season	Year/season interaction
Gap	NS	***	*	*
Chromatid	Deletions	NS	***	*
	Exchanges	NS	***	**
Chromosome	Deletions	NS	***	*
	Exchanges	NS	***	*
Other	NS	***	***	**
Aberrations/100 metaphases	Including gaps	NS	**	NS
	Excluding gaps	NS	***	*
% Aberrant metaphases	Including gaps	NS	***	NS
	Excluding gaps	NS	***	*

NS, not significant ($p > 0.05$).

*Significant, $p \leq 0.05$.

**Significant, $p \leq 0.01$.

***Significant, $p \leq 0.001$.

Taking the eight sampling times overall, mean SCE in 1987 was significantly greater ($p < 0.01$) than mean SCE in 1988. There was a significant effect of sampling ($p < 0.001$), with winter and spring giving a greater mean SCE frequency than summer and autumn. There was a significant interaction ($p < 0.05$) between year and season. This was due to the fact that the differences between corresponding seasons in the 2 years were similar in autumn, winter, and spring but there was no difference between summers. There was no significant effect ($p > 0.05$) of sex overall, and there was no significant interaction ($p > 0.05$) between year and sex or between year, season, and sex.

The results of the evaluation of mitogen-induced blastogenesis are shown in Table 7. There was no significant difference ($p > 0.05$) between males and females at any of the eight sampling times taken individually. Generally, however, even though effects were not significant, the

Table 6. Mean number of sister chromatid exchanges per cell for each sampling time.

Sex	Year 1, 1987				Year 2, 1988				Total
	W	Sp	Su	A	W	Sp	Su	A	
M	7.15	7.18	6.30	7.00	6.26	6.15	6.62	4.34	6.57
F	7.48	7.70	6.30	6.29	6.83	6.50	6.28	5.92	6.83
Total	7.32	7.45	6.30	6.67	6.53	6.29	6.46	5.33	6.69

Abbreviations: W, winter; Sp, spring; Su, summer; A, autumn.

Table 7. Mean maximum response to phytohemagglutinin (dpm) for each sampling time.

Sex	Year 1, 1987				Year 2, 1988				Total
	W	Sp	Su	A	W	Sp	Su	A	
M	42853	50251	33407	34933	26195	20610	15320	15808	31686
F	47380	55362	34316	33655	28613	22004	17152	17374	33971
Total	45116	52807	33849	34280	27468	21307	16267	16504	32829

Abbreviations: dpm, disintegrations per minute; W, winter; Sp, spring; Su, summer; A, autumn.

mean value for the maximum response to PHA tended to be higher in females than in males.

Taking the eight sampling times overall, there was a significant effect ($p < 0.001$) of year, with mean maximum disintegrations per minute in 1987 being higher than in 1988. There was a significant effect ($p < 0.001$) of season, with winter and spring giving a greater mean maximum disintegrations per minute than summer and autumn. There was a significant interaction ($p < 0.001$) between year and season. This was due to the fact that the differences between corresponding season in the 2 years were similar in summer, autumn, and winter but larger in spring. There was no significant effect ($p > 0.05$) of sex overall, and there was no significant interaction ($p > 0.05$) between year and sex or between year, season, and sex.

Table 8 shows SCE frequency in human lymphocytes from four donors in a separate study sampled at shorter time intervals than seasonally. It can be seen that one individual (donor 3) is particularly variable over time.

Discussion

Although previous reports (1-3) showed a significant difference between males and females with respect to CA, SCE, and mitogen-induced blastogenesis, in this study there were no significant differences. There was, however, a nonsignificant increase in SCE frequency in females over

five of the eight sampling times examined. Fifty cells were examined per individual in this and our earlier study. In the case of CA, however, 500 cells per individual were examined in the previous study and only 100 in the present one due to financial constraints. It was not until Margolin and Shelby (6) pooled data from several studies that sex differences could be determined for SCE. The fact that sufficient volume of data is required might equally apply to CA.

We did not find a significant sex difference in the present study in mitogen-induced blastogenesis. In fact, the females showed nonsignificant increases in mitogen-induced blastogenesis in seven of the eight sampling times, but the mean values decreased chronologically. This suggests that such events were independent of seasonal variation because if they were not, levels in the second year would be expected to duplicate those in the first. In fact, such duplication of levels would also be expected for CA and SCE, and this also was not the case.

In order to rigorously control technical conditions, all materials were purchased as single batches before the study began. An earlier study (17) showed that altering only one reagent bromodeoxyuridine had an effect on SCE frequency. The reasons why values for both males and females might have altered with chronological sampling time may be that reagents aged over the 2 years, particularly [3 H]thymidine used in the mitogen-induced blastogenesis assay. Toward the end of the study, fewer cells were available for scoring for SCE and CA.

There was a significant effect of season of sampling taken over both years. In the case of SCE, summer and autumn gave a lower mean SCE frequency than winter and spring. In the case of CA, means obtained in autumn and winter were nearly always lower than those obtained in spring and summer. This suggests an independence of end points.

Seasonal (sampling time) differences have been found in this study but so have differences between years. Sampling time differences have been reported by other workers (27,26). The year differences in the present study, where mean values for the second year are different from

Table 8. Sister chromatid exchange frequency in human lymphocytes from four donors (without treatment).^a

Experiment ^b	Replicate	Donor			
		1	2	3	4
1	1	12.4 ± 6.8	10.5 ± 3.7	5.4 ± 2.6	7.8 ± 3.1
1	2	14.0 ± 4.3	9.6 ± 4.3	4.2 ± 2.0	8.6 ± 3.6
2	1	12.9 ± 4.7	10.4 ± 3.5	6.0 ± 3.1	9.0 ± 3.9
2	2	12.7 ± 3.3	9.5 ± 2.8	7.1 ± 3.3	6.9 ± 2.0
3	1	10.3 ± 3.9	8.1 ± 3.2	9.8 ± 3.1	8.8 ± 4.4
3	2	10.7 ± 3.0	9.2 ± 3.6	13.0 ± 4.3	8.5 ± 4.4

^aBromodeoxyuridine added at 24 hr; cultures harvested at 72 hr; 24 metaphases scored from each culture; mean ± SD shown.

^bExperiment 1, November 5, 1990; experiment 2, January 21, 1991; experiment 3, January 28, 1991.

those in the first year, have no obvious explanation other than that there is no constant pattern due to seasonal sampling.

It can be seen that when individuals are compared over short-term sampling times, one individual has considerably more variability than the others. This may well be the pattern in the population in general.

This present study highlights some of the difficulties in human monitoring. These have previously been outlined by Anderson et al. (1), but some difficulties still need emphasizing. Concurrent controls are essential because the end points examined depend on precise culture conditions, processing, and handling. Even with attempts to rigorously control these conditions, reagents can alter with time. If new reagents are bought at each sampling time, they may be from different batches, and this was shown to be unacceptable (17). Sufficient cells should be examined to ascertain differences. Some lifestyle factors are interdependent (e.g., drinkers tend to smoke and vice versa) and cannot be independently accounted for. However, individuals in control and exposed populations should be carefully matched for as many lifestyle factors as possible.

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