Sister Chromatid Exchange Analysis and Chromosomal Aberration Studies in Interventional Cardiology LABORATORY WORKERS

ONE YEAR FOLLOW UP STUDY

Mustafa Kemal EROL,¹ MD, Sitki OZTAS,² MD,
Engin BOZKURT,¹ MD, *and* Sule KARAKELLEOGLU,¹ MD

SUMMARY

Invasive cardiology laboratory workers are occupationally exposed to chronic ionizing radiation. It is known that ionizing radiation has a damaging effect on chromosomes. In present study, we investigated the frequency of sister chromatid exchange (SCE) and chromosomal aberrations in 11 invasive cardiology laboratory workers and 11 healthy controls. After a vacation period, we took blood samples for chromosome analysis in months 0, 4, 8 and 12 (last two month period was the nonradiation time).

The SCE frequencies did not change significantly after exposure to ionizing radiation in any worker. Our study has revealed that non-specific structural chromosome aberrations such as gaps, isogaps, acentric chromosomes, chromatids and chromosome breakage could be in the 4th and 8th months after ionizing radiation exposure in the metaphase plaques. All abnormal chromosomal effects had disappeared by the end of the two month non-exposure period in each worker.

In conclusion, the results suggest that SCE frequencies are not significantly affected in invasive cardiology laboratory workers who are exposed occupationally to ionizing radiation, although some degree of reversible chromosomal aberrations did appear. (Jpn Heart J 2002; 43: 159-166)

Key words: Sister chromatid exchanges, Chromosomal aberration, Ionizing radiation

SISTER chromatid exchange (SCE) represents the interchange of DNA replication products at apparently homologous chromosomal loci. These exchanges presumably involve DNA breakage and reunion. SCE analysis has come into use as a sensitive DNA damage indicator.¹⁻⁵⁾ Various physical and chemical agents such as ultraviolet light, viral infections, ionizing radiation, smoking, chemotherapeutic

From ¹ Department of Cardiology, ² Department of Medical Genetics, Ataturk University Medical Faculty, Erzurum, Turkey.

Address for correspondence: Mustafa Kemal EROL, MD, Ataturk Universitesi Tıp Fakultesi, Kardiyoloji Anabilim Dalı, 25050 Erzurum Turkey.

Received for publication July 13, 2001.

Revised and accepted December 10, 2001.

tics, and some idiopathic disorders have an increasing effect on the frequency of SCE.⁶⁻⁹⁾

The other method of detecting mutagenic effects is the assessment of chromosomal aberration. Invasive cardiology laboratory workers are exposed to chronic ionizing radiation. It is known that ionizing radiation has a damaging effect on chromosomes. Ionizing radiation is a strong physical mutagen and clastogenic agent, causing the breakage of phosphodiester bonds in DNA.¹⁰⁾ The effects of a wide range of radiations, such as x-rays, gamma-radiation, alpha and beta particles, and neutrons, have been studied in this respect.¹¹⁻¹⁹⁾ Ionizing radiation can cause chromosome breaks at any stage of the mitotic cycle or during meiosis. It is believed a chromosome is broken when it is hit directly by an ion or an ion cluster. There is a very important observation which states that oxygen greatly enhances the effects of radiation, and reducing substances, like those containing sulfhydryl groups, counteract radiation damage.⁵⁾

In this present study, we analysed SCE and chromosomal aberrations in invasive cardiology laboratory workers over a one year period.

MATERIALS AND METHODS

The study was carried out in 11 invasive cardiology laboratory workers (9 males, 2 females; mean age 34.36 ± 3.85 years) and 11 sex-age matched healthy control persons who were not exposed to ionizing radiation. After a two month vacation (non exposure period) SCE and chromosome aberrations were studied 0, 4, 8 and 12 months after ionizing radiation exposure. All workers had a vacation between the 11th and 12th month (non-exposure period).

Heparinised peripheral venous blood samples were used for SCE and chromosome analysis, and two separate cultures were obtained for each sample. Briefly, the blood samples were cultured for 72 hours at 37°C with RPMI-1640 (Sigma) in 20% fetal calf serum (Bio chrome) and 2% phytohemagglutinin M (Sigma) containing 5 mL medium for routine chromosome analysis. For SCE analysis, only 0.1 mL of 10^{-2} M 5-bromo-2-deoxyuridine (Sigma) was added to another culture. Two hours before harvest 0.1 mg colcemid (from 10 mg/mL stock) was added. The cultures were harvested and chromosome preparations were made according to routine protocol.²⁰⁾ Bromodeoxyuridine-incorporated metaphase chromosomes were stained by the fluorescence plus Giemsa technique as described by Wolf and Perry.^{2,20)} Analysis was conducted by selecting 20 satisfactory metaphase plaques for SCE and 50 metaphase plaques for routine chromosome studies.

Statistical Analysis: The results are expressed as the mean $\pm$ SEM. Values at different times in the same group were compared by the Wilcoxon paired *t* test. The SCE results between different groups were analysed by the Mann-Whitney U test. For all analyses, a value of $p < 0.05$ was taken as statistically significant.

RESULTS

The level of ionizing radiation exposure in all cases (2.3 ± 1.7 mSv) was below the accepted annual legal limit (< 50 mSv) set by the National Atomic Energy Association. SCE indexes measured in the study group are presented in Table I. Mean SCE frequencies were 6.67 ± 0.26 at the 4th month ($p = 0.056$), 6.63 ± 0.32 at the 8th month ($p = 0.08$), and 6.57 ± 0.33 at the 12th month ($p = 0.07$) in the study group (Table II). The mean of SCE frequencies was 6.37 ± 0.38 in the study group at the beginning and 6.27 ± 0.38 in the control group ($p > 0.05$). The

Table I. SCE Frequencies in Cases at the Beginning of the Study (0 months) and 4, 8, and 12 Months

Case No.	Occupation	Sex	SCE frequencies per cells (mean $\pm$ SEM)			
			At the beginning	4 th month	8 th month	12 th month
1	Cardiologist	M	5.20 ± 0.17	6.00 ± 0.15	5.30 ± 0.11	5.50 ± 0.11
2	Cardiologist	M	5.80 ± 0.09	6.50 ± 0.15	6.00 ± 0.16	6.20 ± 0.20
3	Cardiologist	M	5.60 ± 0.15	5.75 ± 0.19	5.92 ± 0.12	5.75 ± 0.12
4	Cardiologist	M	5.20 ± 0.16	6.00 ± 0.10	5.30 ± 0.11	5.40 ± 0.11
5	Cardiologist	M	6.00 ± 0.07	6.60 ± 0.12	6.50 ± 0.11	6.30 ± 0.10
6	Nurse	F	5.20 ± 0.09	5.60 ± 0.18	6.20 ± 0.21	5.60 ± 0.21
7	Nurse	F	7.10 ± 0.16	7.50 ± 0.27	7.20 ± 0.29	7.20 ± 0.29
8	Technician	M	7.50 ± 0.15	7.20 ± 0.09	7.10 ± 0.07	7.00 ± 0.15
9	Technician	M	7.20 ± 0.12	7.30 ± 0.15	7.30 ± 0.13	7.60 ± 0.16
10	Technician	M	6.11 ± 0.34	6.50 ± 0.16	7.11 ± 0.16	6.80 ± 0.20
11	Technician	M	9.20 ± 0.16	8.50 ± 0.19	9.00 ± 0.15	9.00 ± 0.13

SCE=sister chromatid exchange.

Table II. Mean SCE Frequencies Measured at the Beginning 0 months and after, 4, 8 and 12 months in the Study Group

Time	SCE	<i>P</i> value
At the beginning	6.37 ± 0.38 (5.2-9.2)	
4 th month	6.67 ± 0.26 (5.6-8.5)	0.056
8 th month	6.63 ± 0.32 (5.3-9.0)	0.08
12 th month	6.57 ± 0.33 (5.4-9.0)	0.07

SCE=sister chromatid exchange.

SCE frequencies were similar in both groups in the 4th, 8th and 12th months also ($p>0.05$) (Table III).

Although chromosome aberrations were not observed in any case at the beginning of the study, all cases had non-specific structural chromosome aberrations such as gaps, isogaps, acentric chromosomes, chromatids and chromosome breakage, in the 4th and 8th months after ionizing radiation exposure in the metaphase plaques (Figure). The frequency of gaps and isogaps was $11.64\pm0.93\%$ in the 4th month ($p=0.003$) and $10.82\pm0.76\%$ in the 8th month ($p=0.003$). The frequency of gaps and isogaps was not statistically different between the 4th and 8th months ($p>0.05$). The frequency of acentric chromosomes, chromatids and chromosome breakage was $4.00\pm0.66\%$ ($p=0.004$) in the 4th month and $3.27\pm0.30\%$ ($p=0.002$) in the 8th month, and the frequencies were not statistically different between the 4th and 8th months ($p>0.05$). All abnormal chromosomal effects had disappeared by the end of the two month non-exposure

Table III. Sister Chromatid Exchange Frequencies Measured in the Study Group and Control Group

Time	Study group (n=11)	Control group (n=11)	P
At the beginning	6.37 $\pm$ 0.38	6.27 $\pm$ 0.38	NS
4 th month	6.67 $\pm$ 0.26	6.33 $\pm$ 0.36	NS
8 th month	6.63 $\pm$ 0.32	6.17 $\pm$ 0.37	NS
12 th month	6.57 $\pm$ 0.33	6.42 $\pm$ 0.19	NS

NS=not significant.

Table IV. Frequency of Gaps and Isogaps in Cases at the Beginning of the Study (0 months) and after 4, 8, and 12 Months

Case No.	Occupation	Sex	Gaps and isogaps (%)			
			At the beginning	4 th month	8 th month	12 th month
1	Cardiologist	M	0	12	10	0
2	Cardiologist	M	0	14	12	0
3	Cardiologist	M	0	8	12	0
4	Cardiologist	M	0	10	8	0
5	Cardiologist	M	0	8	6	0
6	Nurse	F	0	10	10	0
7	Nurse	F	0	16	12	0
8	Technician	M	0	12	10	0
9	Technician	M	0	16	14	0
10	Technician	M	0	8	6	0
11	Technician	M	0	14	10	0
Mean $\pm$ SEM			0	11.64 $\pm$ 0.93*	10.82 $\pm$ 0.76*	0

$p<0.005$

Figure. Chromosome aberrations in three different metaphase plaques (arrows).

Table V. Frequencies of Acentric Chromosomes, Chromatids and/or Chromosome Breakage in Cases at the Beginning of the Study (0 months) and after 4, 8, and 12 Months

Case No.	Occupation	Sex	Acentric chromosome, chromatids and/or chromosome breakage (%)			
			At the beginning	4 th month	8 th month	12 th month
1	Cardiologist	M	0	4	4	0
2	Cardiologist	M	0	4	2	0
3	Cardiologist	M	0	0	4	0
4	Cardiologist	M	0	4	4	0
5	Cardiologist	M	0	4	2	0
6	Nurse	F	0	2	4	0
7	Nurse	F	0	2	2	0
8	Technician	M	0	4	2	0
9	Technician	M	0	6	4	0
10	Technician	M	0	6	4	0
11	Technician	M	0	8	4	0
Mean±SEM			0	4.00±0.66*	3.27±0.30*	0

p<0.005

period (Table IV and Table V). No chromosomal aberrations were detected in the 12 healthy controls.

DISCUSSION

In recent decades, invasive procedures have been increasing in the field of cardiology. Thus, those who work in invasive cardiology laboratories have been occupationally exposed to chronic ionizing radiation. Although protective clothes such as leaded-aprons, and eye glasses are worn by the laboratory workers, they still are exposed to high doses of ionizing radiation. Working under these high dose-radiation circumstances poses a significant risk to their health.

Although a few studies have reported an increase in the frequency of SCE due to ionizing radiation and gamma radiation exposure, other studies have reported that while there were some increases in the frequencies of SCE, the increases were not statistically significant.¹¹⁻¹³⁾ In the present study, we found no significant increase in the frequency of SCE.

It has been pointed out that chromosome aberration frequencies provide the most reliable biological marker of the exposure dose for acute accidental radiation.¹⁴⁾ It is reported that significant radiation-induced changes in the frequency of chromosome aberrations can be detected at very low doses.¹⁵⁾ In this study, we found that the frequencies of chromosome aberrations such as gaps, isogaps, acentric chromosomes, chromatids and chromosome breakage increased after ionizing radiation exposure in the 4th and 8th months. These effects were revers-

ible. After a two month non-radiation (vacation) period there were no chromosomal aberrations. Radiation-induced chromosomal aberrations have been reported in several studies like in our study.¹⁶⁻¹⁹⁾

The results of this study led us to believe that doses of occupationally exposed ionizing radiation in invasive cardiology laboratory workers are ineffective at inducing SCE frequencies, but can produce chromosomal aberrations. This idea is supported by the study of Perry, *et al* in which higher doses of ionizing radiation were able to affect SCE frequencies and chromosomal aberrations. In addition to this, it has been reported that a combination of SCE frequency and chromosomal aberrations could be used in assessment of the mutagenic effects of ionizing radiation.³⁾

In conclusion, the results suggest that SCE frequencies were not affected significantly in invasive cardiology laboratory workers exposed occupationally to ionizing radiation during the year of our study, although some degree of reversible chromosomal aberrations was observed.

REFERENCES

1. Dean BJ, Danford N. Assays for the detection of chemically-induced chromosome damage in cultured mammalian cells. In: Venitt S, Parry JM, Eds. *Mutagenicity Testing, A Practical Approach*. Oxford: Oxford University Press, 1984; 187-232.
2. Wolf S, Perry P. Differential Giemsa staining of sister chromatids and the study of sister chromatid exchange without autoradiography. *Chromosoma* 1974; 48: 341-53.
3. Perry P, Evans HJ. Cytological detection of mutagen-carcinogen exposure by sister chromatid exchange. *Nature* 1975; 258: 121-4.
4. Nakanishi N, Schneider EL. In vivo sister chromatid exchange: a sensitive measure of DNA damage. *Mutat Res* 1979; 60: 329-37.
5. Therman E, Susman M. *Human Chromosomes Structure, Behavior, and Effects*. New York: Springer-Verlag, 1993; 107-17 and 126-34.
6. Benerjee A, Benedict WF. Production of sister chromatid exchanges by various cancer chemotherapeutic agents. *Cancer Res* 1979; 39: 797-9.
7. Rowland RE. Increased sister chromatid exchange in the peripheral blood lymphocytes of young women who smoke cigarettes. *Hereditas* 1999; 131: 143-6.
8. Sonmez S, Senel K, Oztas S, Erdal A, Cerrahoglu L. Sister chromatid exchange analysis in the lymphocytes of patients with ankylosing spondylitis. *Annals of The Rheumatic Diseases* 1997; 56: 275-7.
9. Kaya M, Koçer I, Oztas S. Comparison of sister chromatid exchange frequency between two distinct form of anterior uveitis. *Turkish J Med Sci* 2000; 30: 71-6.
10. Sankaranarayanan K. Prevalence of genetic and partially genetic disease in man and the estimation of genetic risks of exposure to ionizing radiation. *Am J Hum Genet* 1988; 42: 651-62.
11. Gundy S, Varga L, Bender MA. Sister chromatid exchange frequency in human lymphocytes exposed to ionizing radiation in vivo and in vitro. *Radiat Res* 1984; 100: 47-54.
12. Sahin FI, Donmez H, Menevse S. Sister chromatid exchanges in radiodiagnostic unit technicians. *Hamdard Med* 1995; 4, 45-9.
13. Sonmez S, Ikbal M, Yildirim M, Gepdiremen A, Oztas S. Sister chromatid exchange analysis in patients exposed to low doses of iodine-131 for thyroid scintigraphy. *Mutation Research* 1997; 393: 259-62.

14. Brooks AL, Khan MA, Jostes RF, Cross FT. Metaphase chromosome aberrations as markers of radiation exposure and dose. *J Toxicol Environ Health* 1993; 40: 277-88.
15. Bigatti P, Lamberti L, Ardito G, Armellino F. Cytogenetic monitoring of hospital workers exposed to low-level ionizing radiation. *Mutat Res* 1988; 204: 343-7.
16. Balasem AN, Ali AS, Mosa HS, Hussain KO. Chromosomal aberration analysis in peripheral lymphocytes of radiation workers. *Mutat Res* 1992; 271: 209-11.
17. Pazy-Mino C, Leone PE, Chavez M, *et al*. Follow up study of chromosome aberrations in lymphocytes in hospital workers occupationally exposed to low levels of ionizing radiation. *Mutat Res* 1995; 335: 245-51.
18. Stephan G, Hadnagy W. Chromosomal aberrations in patients exposed to x-rays and contrast medium. *Eur J Radiol* 1981; 1: 335-7.
19. Kumagai E, Tanaka R, Kumagai T, Onomichi M, Sawada S. Effects of long-term radiation exposure on chromosomal aberrations in radiological technologists. *J Radiat Res* 1990; 31: 270-9.
20. Rooney DE, Czepulkowski BH, *Human Cytogenetics: A Practical Approach*. Lancaster IRL Press, 1992; 31-46 and 106-110.