Radiol Oncol 2005; 39(2): 153-8.

Cytogenetic analysis of peripheral blood lymphocytes after arteriography (exposure to x-rays and contrast medium)

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Backgrounds. *The purpose of our study is to investigate the cytogenetic analysis findings in peripheral blood lymphocytes of 29 patients who had undergone diagnostic radiography.*
Methods. *Peripheral blood samples were taken from 22 patients submitted to renal arteriography and 7 patients submitted to cerebral arteriography (17 male and 12 female, aged between 13-68 years). Cytogenetic analyses of peripheral lymphocytes were performed before the procedure, immediately after and 24 hours later. The entrance skin dose obtained during the whole diagnostic X-ray exposure was measured by thermoluminescent dosimeters and varied between 0.03-0.30 Gy. Both low and high osmolarity contrast media were used. Chromosomal aberrations and micronuclei frequency were used as biomarkers of genotoxicity.*
Results. *The estimated frequency of chromosomal aberrations and micronuclei in the peripheral blood lymphocytes of patients after arteriography examination was significantly higher than the level before the diagnostic exposure. The mean frequency of cells with chromosomal aberrations was nearly double after examination and proved to be constant in the analysis after 24 hours.*
Conclusions. *Radiological diagnostic procedures involving iodinated contrast media as arteriography may cause a significant increase in cytogenetic damage in peripheral blood lymphocytes.*

Key words: angiography – adverse effects; lymphocytes; chromosome aberrations; micronucleus tests

Introduction

Iodinated contrast media are largely needed in diagnostic radiology. In angiography and interventional radiology, especially high diagnostic doses are obtained - relatively long fluoroscopy time plus serial radiography (several frames per second). Cytogenetic analysis findings of diagnostic doses of ×-rays and contrast media were investigated in experimental studies on cell cultures *in vitro*.[1,2] Parallel clinical investigations showed an increased genotoxicity in the peripheral blood lymphocytes of the patients undergoing angiography.[2-4] The results indicate that some contrast media can

Received 17 October 2004
Accepted 11 November 2004

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induce genotoxic effects alone, and when applied in combination with X-rays, can increase, even double the radiation induced genetic damage. Radiological contrast media do not only increase the absorbed dose, but may also enhance the sensitivity of blood cells to the radiation induced cell damage.[2-4]

Cytogenetic analysis results are of great concern as they are involved in the mechanism of cancer genesis. It is generally accepted that chromosomal mutations are causal events in the development of neoplasia and it has been postulated that an increased cytogenetic damage may be an indication of an enhanced cancer risk.[5]

The aim of the present study is to investigate the effects of contrast media and diagnostic radiation on cytogenesis of the peripheral blood lymphocytes of the patients undergoing arteriography. Chromosomal aberrations (CA) and micronuclei (MN) in the peripheral blood lymphocytes are used as cytogenetic biomarkers.

Methods

Subjects investigated

Twenty-nine patients with limited history of previous medical radiation exposures and undergoing angiography examination [22 renal arteriographies (RAr) and 7 cerebral arteriographies (CAr)] were selected for this study. In the selected group of patients, 17 were males and 12 females, ranging in age from 13 to 68 years (average age 41.6 years).

A Philips Medical Systems angiographic equipment »PolyDiagnost C« was used with DSI viewing console and Easy Vision workstation. The unit was operated at 60 - 90 kV range and up to 250 mA with a filtration of 2 mm Al.

Blood samples were collected in sterile vacationers with Li-heparin. Three samples were taken: (1) before angiographic run, (2) immediately after, and (3) 24 hours after the examination. The radiation exposure assessment was made by thermoluminescent dosimeters. The radiation exposure varied from 0.03 to 0.30 Gy (Table 1) and was estimated as skin entrance dose. The type and the volume of contrast material used are given in Table 1. For all subjects, a questionnaire was completed to assess their general physical condition, life style, previous ×-ray examinations, diets, use of medications.

Cytogenetic endpoints

Lymphocyte cultures were prepared in 5 ml RPMI-1640 medium supplemented with 10% fetal calf serum and phytochaemaglutinin P.

For chromosomal aberration analysis, Colchicine 0.5 mkg/ml was added to the cultures 48 hours after incubation. The cells were harvested two hours later.[6] Twenty-eight subjects were analyzed for chromosomal aberrations (CA). The cells scored per sample for structural chromosomal aberrations after staining with 10% Giemsa ranged from 100 to 400.

For cytokinesis blocked micronucleus test, Cytochalasin B was added 44 hours after incubation. The cells were harvested after 72 hours (7). Ten patients were analyzed for the presence of micronuclei (MN) in binucleated lymphocytes immediately before (1) and after (2) radiodiagnostic examination. Two thousand cells per each sample were analyzed.

Ethics

Informed consent was obtained from all investigated subjects after they had received an explanation of the study. The reports were reviewed and approved by the local ethics committee. The volume of the samples (1) and (2) is the blood collected during the air trapping prevention and catheters flushing.

Statistical analysis

Student t- test and χ^2- test was applied before and after arteriography of patients to analyze

statistical significance of the difference between the frequencies of chromosomal aberrations and micronuclei formation, respectively.

Results

A total of 29 subjects submitted to angiography were investigated cytogenetically. Chromosomal aberrations were analyzed in 28 of them, and in 10 subjects, micronuclei formation in binucleated lymphocytes was investigated (Table 2).

The frequency of chromosomal aberrations was increased in most of the patients immediately after the examination and remained constant at the sampling after 24 hours (Table 2). Dicentric chromosomes, which are the most sensitive indicators of radiation exposure, were found in 7 cases. It must be noted that, despite selection, some of the patients underwent some kind of radiodi-

Table 1. Characteristics of the investigated patients undergoing arteriography

Nº	Case	Age	Sex	Smoker	Type* of examination	Contrast agent (mg J/ml)	Total volume (ml)	Entrance skin dose (Gy)	Ro-exam. in last 1 year	Sampling time** CA	MN
1.	GI	28	M	yes	CAr	iodixanol 320	80		Head CT	1; 2	1; 2
2.	KG	45	F		CAr	iodixanol 320	80		Head CT	1; 2	1; 2
3.	DG	56	F	no	CAr	iopromide 300	50		Head CT	1; 2	1; 2
4.	RF	44	F		RAr	iopromide 370	40		IVU	1; 2	1; 2
5.	DS	39	M	yes	RAr	iohexol 350	40		no	1; 2	1; 2
6.	SM	49	M	yes	CAr	iohexol 350	50		Head CT	1; 2	1; 2
7.	DK	36	M	yes	CAr	iohexol 350	50		Head CT	1; 2	1; 2
8.	ED	64	F	no	CAr	iohexol 350	50		Head CT	1; 2	
9.	MP	50	M		CAr	iopromide 300	80		Head CT	1; 2	1; 2
10.	PD	35	M	yes	RAr	diatrizoate 370	58	0,09	IVU	1; 2; 3	
11.	II	65	F	no	RAr	diatrizoate 370	52	0,08	Abdominal	CT	1; 2; 3
12.	EL	60	F	no	RAr	ioxaglate 320	50	0.20	IVU	1; 2; 3	
13.	PX	42	M		RAr	diatrizoate 370	46		no	1; 2; 3	
14.	HI	33	M	no	RAr	diatrizoate 370	46	0,20	no	1; 2; 3	
15.	SV	13	F	no	RAr	diatrizoate 370	48	0,19	IVU	1; 2; 3	
16.	HS	17	F	no	RAr	diatrizoate 370	18	0,05	no	1; 2; 3	
17.	ML	33	F	no	RAr	diatrizoate 370	50	0,15	no	1; 2; 3	
18.	VI	18	M	no	RAr	diatrizoate 370	60	0.3	IVU	1; 2; 3	
19.	TG	38	F	no	RAr	diatrizoate 370	40	0.03	no	1; 3	
20.	PP	58	M	yes	RAr	diatrizoate 370	30	0.11	RA	1; 2; 3	
21.	AD	29	M	no	RAr	diatrizoate 370	36	0.15	no	1; 2; 3	
22.	VY	68	M	no	RAr	iopromide 300	50	0.19	no	1; 2	1; 2
23.	DZ	46	M	yes	RAr	diatrizoate 370	50		no	1; 2; 3	
24.	SD	44	F		RAr	diatrizoate 370	35	0.26	IVU	1; 2	
25.	ID	21	M	yes	RAr	diatrizoate 370	40	0.08	no	2; 3	
26.	ME	68	F		RAr	diatrizoate 370	14		Abdominal CT; IVU	1; 2	
27.	GV	64	M		RAr	diatrizoate 370	45			1; 2	
28.	HP	52	M		RAr	diatrizoate 370	45			1; 2	
29.	TZ	34	M	no	RAr	iopromide 300	50	0.03	no	1; 2	

* CAr - Cerebral arteriography, RAr - Renal arteriography; **1 - before arteriography, 2 - after arteriography, 3 - 24 hours after arteriography

Table 2. Frequency of chromosomal aberrations (CA) and micronuclei (MN) in the peripheral blood lymphocyte of the patients undergoing to arteriography

		Chromosomal aberrations, %								
№ Case	Sampling time*	CA, № scored cells	Cells with CA, %	Chromosome Fragments	Dicentrics	Chromatide Fragments	Total № of CA, %	MN, ? scored cells	Cells with MN, ‰o	Total № of MN,%
1. GI	1.	200	2	1	0	1	2	2000	16.00	19.5
	2.	250	2,8	2,4	0	0,4	2,8	2000	24.00	27.00
2. KG	1.	200	1	1	0	0	1	2000	8.00	8.00
	2.	200	1	1	0	0	1	2000	13.00	15.00
3. DG	1.	200	1	0,5	0	0,5	1	2000	14.50	17.00
	2.	200	0,5	0,5	0	0	0,5	2000	14.00	15.50
4. RF	1.	200	0.5	0	0	0,5	0,5	2000	7.00	7.00
	2.	200	1	1	0	0	1	2000	9.00	9.50
5. DS	1.	200	1	0	0	1	1	2000	7.00	7.00
	2.	200	2	1,5	0	0,5	2	2000	5.50	6.00
6. SM	1.	200	1,5	1	0	0,5	1,5	2000	7.00	7.00
	2.	200	1,5	1	0	0,5	1,5	2000	11.50	11.50
7. DK	1.	200	1	0,5	0	0,5	1	2000	5.00	5.00
	2.	200	1,5	0,5	0,5	0,5	1,5	2000	8.00	10.00
8. ED	1.	200	1	0	0	1	1			
	2.	200	0,5	0	0	0,5	0,5			
9. MP	1.	200	0,5	0	0	0,5	0,5	2000	7.00	7.00
	2.	200	0,5	0,5	0	0	0,5	2000	10.00	11.5
10. PD	1.	200	4.5	3	0.5	1	4.5			
	2.	200	3	1.5	0	1.5	3			
	3.	100	5	2	1	2	5			
11. II	1.	200	3	3	0	0.5	3.5			
	2.	400	6	4.25	0.25	1.75	6.25			
	3.	200	4.5	6	0.5	0	6.5			
12. EL	1.	200	2	1	0	1	2			
	2.	200	3	1.5	0	1.5	3			
	3.	100	3	2	0	1	3			
13. PX	1.	100	1	0	0	1	1			
	2.	400	3.25	1.75	0	1.5	3.25			
	3.	200	3.5	2.5	0	1	3.5			
14. HI	1.	200	2.5	2	0	0.5	2.5			
	2.	200	3	2	0	1	3			
	3.	200	5	3.5	0	1.5	5			
15. SV	1.	100	1	1	0	0	1			
	2.	300	2.3	0.7	0	1.6	2.3			
	3.	200	4	1	0	3	4			
16. HS	1.	200	1.5	1	0	0.5	1.5			
	2.	400	4	1.5	0.5	2	4			
	3.	200	3.5	3	0	0.5	3.5			
17. ML	1.	200	1	1	0	0	1			
	2.	200	5.5	4.5	0	1	5.5			
	3.	200	2	2	0	0	2			
18. VI	1.	200	1.5	0.5	0	1	1.5			
	2.	200	2	1.5	0	0.5	2			
	3.	200	3	1.5	0.5	1	3			
19. TG	1.	200	1.5	1	0	0.5	1.5			
	3.	200	2	1	0	1	2			
20. PP	1.	200	1	0.5	0	0.5	1			
	2.	200	1.5	1	0	0.5	1.5			
	3.	200	3.5	2.5	0	1	3.5			

Nº Case	Sampling time*	CA, Nº scored cells	Cells with CA, %	Chromosome Fragments	Dicentrics	Chromatide Fragments	Total Nº of CA, %	MN, ? scored cells	Cells with MN, ‰	Total Nº of MN,%
21. AD	1.	200	0.5	0.5	0	0	0.5			
	2.	200	1	0.5	0	0.5	1			
	3.	200	0.5	0.5	0	0	0.5			
22. VY	1.	200	1.5	1	0	0.5	1.5	2000	9.5	13.5
	2.	400	3.75	3.25	0	2.5	5.75	2000	12.5	15.5
23. DZ	1.	100	0	0	0	0	0			
	2.	200	3.5	1.5	0	2	3.5			
	3.	100	3	2	0	1	3			
24. SD	1.	100	5	3	0	2	5			
	2.	100	4	2	0	2	4			
25. ID	2.	200	3.5	2	0	1.5	3.5			
	3.	200	6.5	3.5	1	2.5	7			
26. ME	1.	200	3	1.5	0.5	1	3			
	2.	300	4.7	2.7	0.3	1.7	4.7			
27. GV	1.	200	2	1	0	1	2			
	2.	200	2	1.5	0	0.5	2			
28. HP	1.	200	2	0.5	0	1.5	2			
	2.	200	1.5	1	0	0.5	1.5			
29. TZ	1.							2000	14	15
	2.							2000	17.5	17.5

*1 – before arteriography, 2 – after arteriography, 3 - 24 hours after arteriography

agnostic examination within the year before entering the study (Table 1).

The mean frequency of cells carrying chromosomal aberrations in the group of 28 investigated patients was 1.62% ± 0.18 before angiography, and 2.77% ± 0.21 immediately after diagnostic examination (Figure 1). The difference was statistically significant (t = 3.21; PZZZ0.01). The frequency of cells with aberrations was estimated 24 hours after the diagnostic exposure only in 14 subjects and was found to be 3.61 % ± 0.39. The frequency score for the same subjects immediately after angiography was 3.39 % ± 0.32 and did not differ significantly in the analysis after 24 hours (PVVV0.05). In the group of patients submitted to renal arteriography, the frequency of cells with chromosomal aberrations immediately before and after the exposure was 1.81% ± 0.22, and 3.22% ± 0.25, respectively (Table 2), (PZZZ0.01). No increase in the frequency of chromosomal aberrations was observed in the patients who has undergone cerebral arteriography (PVVV0.05).

The yield of micronuclei also increased significantly after angiography (Table 2). The frequency varied from 5‰ to 16‰ in subjects before, and from 5.5‰ to 24‰ in different subjects immediately after the examination. The mean values of micronuclei in peripheral lymphocytes of the investigated subjects was 9.5Č ± 0.69 before, and 12.5 ± 0.80 after the examination (Figure2). The difference was statistically significant ($\chi^2 = 7.85$; PZZZ0.01).

Discussion

In this study, we found a higher frequency of chromosomal aberrations and micronuclei in the group of patients exposed to the diagnostic x-ray with the application of contrast media during angiography compared to their control values before the exposure. The difference was statistically significant for both cytogenetic biomarkers used: chromosomal aberrations (PZZZ0.01) and micronuclei formation (PZZZ0.01). Micronuclei arose in the cytoplasm of binucleated cells as a result of CA induction[7] and they were proved to be a sensitive bioindicator of genotoxic exposure.

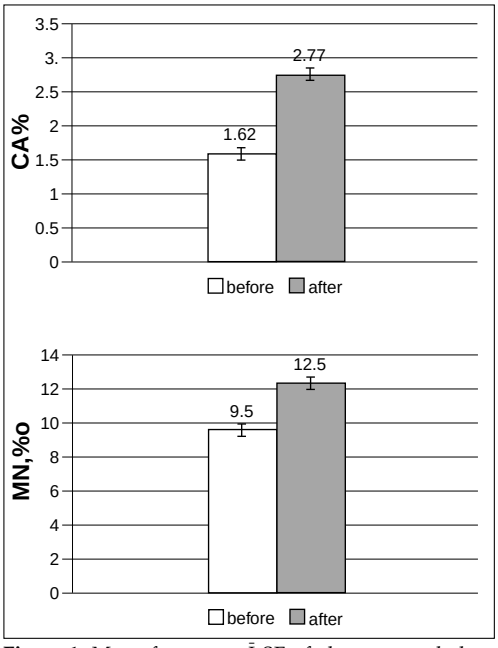

Figure 1. Mean frequency Ī SE of chromosomal aberrations (CA) and micronuclei (MN) in the patients immediately before and after arteriography.

The use of contrast agent in radiodiagnostic arteriography aimed to increase the absorption of X-rays in blood vessels. This was due to the iodine atom included and resulting effect of high photoelectric absorption. As a consequence, the cells in the vicinity of the contrast agent might have absorbed larger radiation dose and might have been exposed to greater cytotoxic effects.[4] This could explain the observed significant genotoxic damage in the peripheral blood lymphocytes of the investigated patients in our study.

Previous *in vitro* studies found that some contrast agents might possess genotoxic properties by themselves[1] and might have a potential to increase the genotoxicity of X-rays as well.[2,4] Previous studies also proved that certain contrast media could also penetrate the epithelial cells through a transcellular mechanism.[8,9]

In conclusion, there is a significant increase in the frequency of chromosome damage in the peripheral blood lymphocytes of the subjects undergoing diagnostic arteriography. These results suggest the need for studying the radiosensitizing property of the contrast media to reduce the patient dose without compromising the image quality. Further *in vitro* studies are needed to elucidate the mechanism of the combined genotoxic effects of iodinated contrast agents and radiation.

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