12. IMPACT OF GENETIC TRAITS ON THE OCCURRENCE OF COLORECTAL CANCER

Response to the Challenging Dose of X-rays in Lymphocytes of Colorectal Cancer Patients and Healthy Donors

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Abstract

An individual's genetic constitution and lifestyle, e.g., diet and levels of physical activity, can affect the body's response to various exogenous agents including therapeutic treatments. The aim of our study was to compare on molecular and cellular levels, responses to the X-rays of peripheral blood lymphocytes from colorectal cancer patients (CCP) with the results from a group of cancer free controls. We wanted to find out if their molecular and cellular radiosensitivity can be associated with predisposition to cancer induction or predict efficiency of therapeutic treatment.

On the molecular level, the alkaline version of the single cells gel electrophoresis (SCGE) assay, also known as a Comet assay, was used to estimate the DNA damage and repair efficiency. The results were compared with the findings of classical cytogenetic

studies on chromosome and chromatid type aberrations. On average, no significant difference neither in susceptibility to the challenging dose, nor in the DNA repair efficiency was found between investigated groups, although DNA repair efficiency was significantly lower than that observed for cells from healthy donors. The findings correlated with the results from cytogenetic studies. Higher amounts of chromosome and chromatid aberrations were detected in irradiated lymphocytes from colorectal cancer patients than in lymphocytes from controls, although the difference between investigated groups was statistically insignificant. Our previous results, suggested a possible value of the DNA repair in epidemiology as a potentially useful test for prediction of cancer predisposition and in pre-clinical studies as a predictor for patient's response to therapy, and radiotherapy in particular. As results of the studies have shown some variations due to gender and lifestyle factors (smoking history, sport activities) further investigations are needed to confirm those observations in larger group of patients.

Introduction

Colorectal cancer (CRC) is one of the most commonly occurring malignancies in both men and women (18). The poor prognosis for patients with advanced CRC has led to the intensive search for new technologies to enable earlier diagnosis and improve treatment results. The study by Richter et al. summarized chromosomal changes detectable in nonpolypoid adenomas of the colon. The authors have demonstrated the presence of distinct differences between nonpolypoid and polypoid adenomas, which supported observations from the earlier genetic and microsatellite studies, that different genetic pathways for tumor progression may exist for colorectal neoplasms of polypoid and nonpolypoid phenotype. Their study stimulated further search for the new gene alterations in nonpolypoid adenomas. The identification of molecular biomarkers specific to early and late events in colorectal cancer progression might be critical for the development of preventive strategies and improve disease detection. On the other hand, biomarker of patient cellular sensitivity (i.e., DNA or cellular response to genotoxic agents) might be used as a prognostic predictor in treatment planning. Chromosome damage in human lymphocytes, very well known biomarker in human monitoring, was first used in 1962 to examine the risk associated with radiation accidental exposure (13, 17). Recent studies (11, 13) have shown positive and statistically significant association between chromosomal damage evaluated in peripheral lymphocytes by classic cytogenetics and subsequent cancer risk. Chromosomal aberrations in peripheral blood lymphocytes are the only biomarkers that can provide information on the dose of exposure and associated risk of cancer, and they have been measured extensively in various circumstances. (1, 11). Recently introduced alkaline version of the comet assay (syn: single cell gel electrophoresis assay, SCGE assay) enables the assessment of genetic damage induced *in vitro* and *in vivo* in a great variety of cells (2, 15). In our study we compared responses to the challenging dose of X-rays of the lymphocytes collected from colorectal cancer patients and free of cancer controls on the molecular and mitotic levels using classic cytogenetics and DNA damage analysis by single cell gel electrophoresis assay (SCGE).

Materials and methods

The study group consisted of 37 colorectal cancer patients (CCP), 17 females (average age 59.7) and 20 males (average age 62.6) and a group of 41 controls with various gastrointestinal disorders without cancer diagnosis (22 females, average age 55.5, and 19 males, average age 58.5). Peripheral whole blood samples were collected into heparinized tubes and transported immediately to the laboratory of the Department of Radiation and Environmental Biology, Institute of Nuclear Physics, Polish Academy of Science. Information about donor's health, lifestyles, diet and habits were collected through the questionnaires. In the study group there were 15.7% recent male smokers, 16.9% former smokers, and 14.5% non-smokers, and among women there were 14.5% recent smokers, 8.4% former smokers, and 24.1% non-smokers.

Experimental procedure

Immediately after collecting blood for testing, the whole blood sample from each subject was divided for culturing and molecular studies.

DNA repair competence assay

For molecular studies, lymphocytes were isolated from the whole blood sample and cryopreserved for further treatment at $-80°C$. Before the beginning of the experimental procedures cells were thawed according to the standard procedure and their viability was examined (2, 7). To evaluate individual susceptibility and repair competence, the extent of DNA damage was studied, using the alkaline version of the single cell gel electrophoresis (SCGE) assay as described elsewhere (2), in defrosted lymphocytes, then after the irradiation, and after post irradiation incubation.

Irradiation of isolated lymphocytes

Isolated lymphocytes were irradiated with 3 Gy dose of X-rays from Philips MCN 323 machine at 250 kV, 10mA. The dose rate applied was 1 Gy/min. The probes for DNA radiosensitivity studies were irradiated on a microscopic slides and the cells for DNA repair studies were irradiated simultaneously in the Eppendorf vials. To avoid DNA repair process during irradiation, the temperature was maintained at below 4°C, and both, microscopic slides containing cells embedded in agarose and Eppendorf vials were positioned on a special polyethylene box containing ice cubes in water. Immediately after irradiation, microscopic slides with irradiated cells were immersed in lysing solution and cells that were irradiated in Eppendorf vials were placed in the box containing ice water bath. Then they were immediately transported to the laboratory, where cells irradiated in the Eppendorf vials were suspended with the 1 ml of medium (80% RPMI and 20% FBS) and transferred into an incubation chamber (37°C) for a period of 40 minutes, time specified in the DNA repair kinetics studies (2) as sufficient to complete fast repair in G_0 cells. After incubation cells were subjected to further steps of SCGE assay as described elsewhere (7) and residual (not repaired during the incubation) DNA damage was detected.

Detection of the DNA damage

The automatic analysis of the DNA in the comets was done using two parameters from the Komet 3.0 or Komet 5.5 software (Kinetic Imaging Company, Liverpool, UK):
- TDNA – Tail-DNA (percentage of DNA in the comet tail).
- TM – tail moment (which expresses the percentage of DNA in the tail multiplied by the tail length (TL) (TL means in our study the length of the comet tail measured from the edge of the comet head, the calibrated unit of tail length is about 0.862 μm).

The result of the analysis was recorded as an average of two independent repetitive and independent electrophoreses performed by two researchers using Komet 3.0 or 5.5 systems. For each experimental point, 100 cells were analyzed (25 from each of two different areas of a slide, together 50 cells from each slide). For analysis, SPSS and the statistical packages from MS Excel or Origin 7.0 software were applied.

To compare cellular DNA repair competence between individuals, DNA damage repair efficiency (RE) was defined as percent of DNA damage that was repaired during the post irradiation incubation, and it was presented as RE_{T-DNA} or RE_{TL}, for the DNA damage detection with the use of % of the DNA in the comet or comet tail length TL measures, and as RE_{TM} with the use of comet tail moment (TM) measures.

Internal standard (healthy donor cells)

To compare cellular capacities of patients and healthy donors, and to control stability of the experimental conditions and to avoid any influence of unpredictable factors on the quality of the final conclusions, an internal standard was put into operation as described earlier (7). Briefly, in each experiment and electrophoresis, a group of cells belonging to the same pool of cells from one sampling probe collected from a healthy male donor called 'Mister Standard' (MS), were subjected to the general procedure as the internal standard (IS). The male donor "MS" was selected as a representative from the historical control group investigated in previous studies (donors reporting none of health problems) (8). Standardizing factors were evaluated with respect to each electrophoresis (i) as the ratio of the results obtained from the response of standardizing cells to the challenging dose in that particular electrophoresis (i) (described as $T\text{-}DNA_{3Gy(i)}$) to the average obtained from the results of the IS cells ($T\text{-}DNA_{3Gy(av)}$) from all experiments in those series of study. This resulted in a standardizing factor (SF) for experiment (i) estimated as follows: $SF_{(i)} = T\text{-}DNA_{3Gy(i)} T\text{-}DNA_{3Gy(av)}$. Then, all the results in each experiment (i) where divided by adequate standardizing factor $SF_{(i)}$ and presented after standardization (7).

Irradiation of whole blood samples

A part of whole blood samples was settled in the box containing ice water bath and was treated as control. The other whole blood probes, placed in the heparinized small specimens tube, were irradiated with 2 Gy dose of X-rays using Philips MCN 323 machine at 250 kV, 10 mA. The dose rate applied was 1 Gy/min. To avoid DNA repair during irradiation all blood samples were placed on a polyethylene box containing ice cubes in

water. Immediately after irradiation, tubes with irradiated blood and not irradiated blood samples were again placed in the box containing ice water bath, transferred to the laboratory and subjected to culturing cytogenetic procedures.

Classic technique – cytogenetics study

Classic cytogenetics culturing method used in the study was based on the procedures that are commonly used for monitoring genotoxic exposures, or for estimating a radiation dose absorbed by subjects involved in the radiation accidents in radiological protection laboratories (3, 6, 5, 12). For classic cytogenetics two probes/repetitions for chromosome aberrations (CA) and two probes for sister chromatid exchanges (SCE) were applied, another four probes were used for fluorescence *in situ* hybridization (FISH) technique (two for irradiated cells and two for controls). All blood samples were incubated at 37°C using RPMI 1640 medium supplemented with 20% fetal calf serum, antibiotics, and various amounts of 5-bromo-2-deoxyuridine (BrdU), (0.05 mM for CA 0.075 mM for SCE and none for FISH techniques). All lymphocytes were stimulated with phytohemagglutinin PHA. Cultures designed for chromosome aberrations analysis were lasting 48 hours, and 72 hours for SCE and FISH techniques. Two hours before the end of culturing, 0.1 µl/ml of colcemid solution was added to the culture to stop cells dividing in a proper stage of metaphase. Then, cells were fixed and stained with fluorescence plus Giemsa solution (for SCE and chromosome preparations) following a standard procedure described elsewhere (17). Slides designed for further study with FISH technique were transferred to –20°C to store before the hybridization procedure with specific molecular and chromosome probes.

For screening of chromatid and chromosomal aberrations, only cells with identically dark-stained chromatids proving a first mitotic division were analyzed (6). Each good metaphase spread of lymphocytes was analyzed in the first mitosis for the presence of unstable chromosome aberrations (CA) and in the second division for sister chromatid exchanges. The frequency of chromosome aberrations was screened in at least 50–100 good metaphase spreads (the number depending on the frequency of aberrations detected). All types of chromosome damage were scored including gaps, breaks in chromosomes and chromatids, fragments and chromatid exchanges. Based on these measurements frequency of chromosome (CSA) and chromatid (CTA) aberrations were evaluated (1). For the analysis of the SCE frequency, 50 metaphases in the second cell division were scored. PRI-proliferate rate index was evaluated from distributions of cells scored in the first (M_1), second M_2 and third division (M_3) according to the following formula: $PRI = (M_1 + 2xM_2 + 3xM_3) / (M_1 + M_2 + M_3)$.

Statistics

Analysis of results was performed using Anova from statistical package for Social Science (SPSS).

Results and Discussion

In the Table 12.1 are shown mean values of cellular capacities evaluated for colorectal cancer patients (CCP = 1) and controls (CCP = 0), based on cells response to challenging dose of radiation and detection of chromosome and chromatid aberrations in the first mitotic division, stratified according to cancer diagnosis followed by stratification according to the gender. Frequency of sister chromatid exchanges (SCE) and mitotic index, shown as PRI were measured in the cells without challenging radiation treatment.

Table 12.1. Mean values of cellular capacities evaluated for groups of colorectal cancer patients (CCP = 1) and controls (CCP = 0) on the basis of cells response to challenging dose of radiation and detection of chromosome and chromatid aberrations in the first mitotic division stratified according to cancer diagnosis, followed by stratification to the gender and cancer diagnosis. Frequency of sister chromatid exchanges (SCE) and mitotic index that are shown as PRI are measured in the cells without challenging radiation treatment

G	CD	n	Age ±SD	AbC ±SD	CTA_{1F} ±SD	CTA_{2F} ±SD	CTA ±SD	CSA ±SD	SCE* ±SD	PRI* ±SD
All	0	41	56.9	49.4	3.17	69.0	72.9	37.0	4.54	2.22
			13.14	10.66	2.82	19.71	20.80	12.28	1.21	.38
	1	37	61.3	51.3	2.90	70.2	75.5	35.7	4.47	2.39
			8.41	9.08	2.54	17.46	18.05	11.66	.97	.41
M	0	19	58.5	47.0	3.52	65.7	70.2	35.1	3.78	1.99
			13.27	11.76	3.29	21.99	23.65	13.24	1.11	.284
	1	20	62.6	49.3	2.67	68.7	71.8	37.5	3.83	2.45
			6.72	9.30	2.23	21.15	21.27	11.25	.75	.29
F	0	22	55.5	51.6	2.86	72.1	75.5	38.7	5.11	2.57
			13.17	9.28	2.36	17.31	17.97	11.45	.99	.15
	1	17	59.7	53.8	3.20	74.9	79.7	36.8	4.96	2.36
			10.40	8.85	2.96	12.44	12.98	9.99	.94	.52

G – gender, M – males, F – females, CD – Cancer diagnosis: 0 – no cancer, control group, 1 – colorectal cancer patients group, AbC – percent of aberrant cells, CSA – percentage of chromosome aberrations, CTA1F – percentage of breaks in the one single chromatid, CTA2F – percentage of acentric fragments from both chromatids, CTA – percentage of all chromatid aberrations, SCE – sister chromatid exchanges frequency per cell, PRI – mitotic index [PRI= (M1 + 2M2 + 3M3) / (M1 + M2 + M3).

* No treatment.

In the whole group, colorectal cancer patients, both men and women, are slightly (about 4 years) older than controls. On the whole statistically no significant differences were observed in all cytogenetic endpoints between CCP and controls, however, differences between groups were slightly increasing after stratifying according to gender. The rate of aberrant cells in CCP group is slightly higher than in the controls (in the whole group, and in the both male and females subgroups). No difference between cancer patients and control groups were observed, without or with stratification according to gender in the percent of breaks in the one single chromatid (CTA_{1F}). Higher in CCP than in control frequencies of chromatid type aberrations that means acentric fragments (CTA_{2F})

and all chromatid type of aberrations (CTA) were observed in the studied group as the whole, and in the both male and females subgroups.

There are no significant differences between cancer patients and controls in the frequency of sister chromatid exchanges detected in the second mitotic division, neither without, nor after gender stratification. However, in general sister chromatid exchanges (SCE) among females are higher than in males, and it can be associated with less efficient homologous recombination in females lymphocytes (16).

Results of the rate of aberrant cells, CSA, CTA and SCE (Fig. 12.1) show comparison between subgroups of cancer patients and controls separately for subgroups of males and females. The slight difference between CCP and controls or males and females is only visible in the frequency of chromatid breaks.
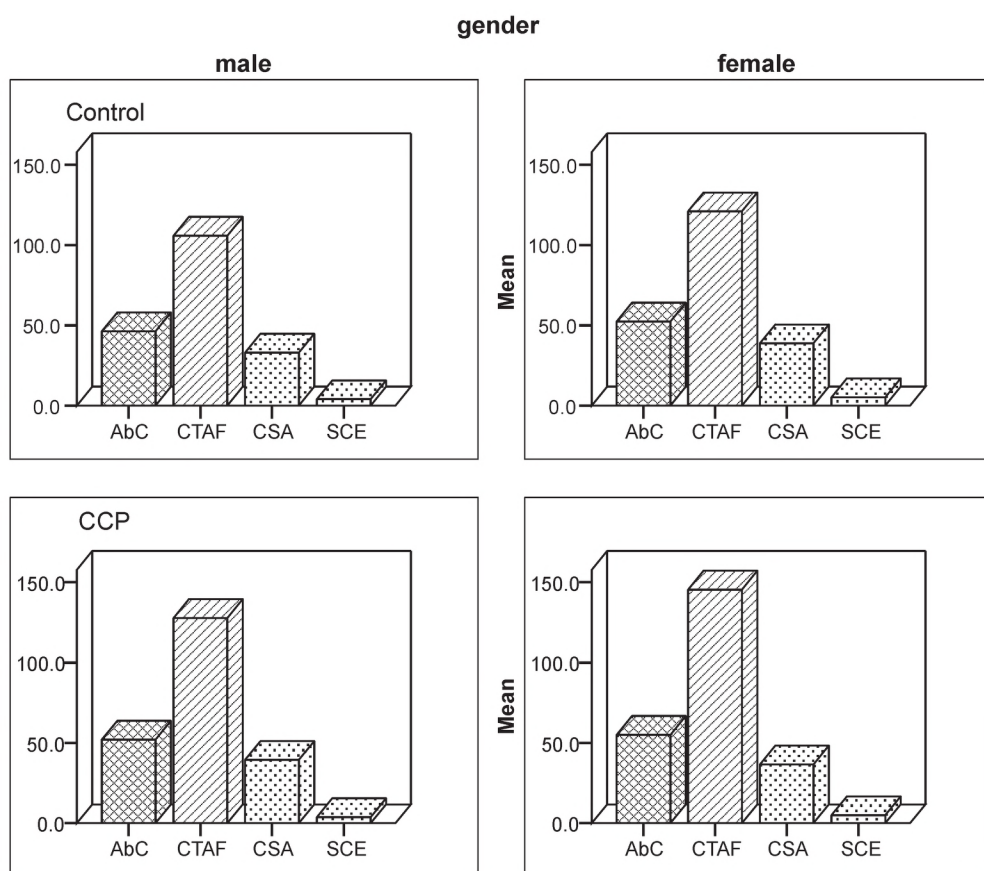

Figure 12.1. Mean values of cellular capacities evaluated for groups of colorectal cancer patients (CCP = 1) and controls (CCP = 0) on the basis of cells response to challenging dose of radiation and detection of chromosome and chromatid aberrations in the first (AbC, CTAF, CSA) or second mitotic division, then stratified according to gender followed by stratification according cancer diagnosis. Frequency of sister chromatid exchanges (SCE) are measured in the cells without challenging radiation treatment

Table 12.2 shows mean values of the same biomarkers of cellular capacities evaluated for investigated groups based on cells response to challenging dose of radiation, as shown in the Table 12.1. The results are stratified according to cancer diagnosis in the immediate family (CiF) then according to gender and finally colorectal cancer diagnosis. After stratification of the study group according to CiF factor and then to cancer diagnosis it appeared that five persons, who reported having relatives with documented cancers were all cancer patients. The number of patients, who report cancer in immediate family (CiF factor) is fairly low, and therefore it is unlikely to find statistically significant influence of genetic predisposition, nevertheless, we observed in these cases higher frequency of chromatid type aberrations and slightly lower frequency of chromosome damage (dicentics and rings).

Table 12.2. Mean values of cellular capacities evaluated for groups of colorectal cancer patients (CCP = 1) and controls (CCP = 0) on the basis of cells response to challenging dose of radiation and detection of chromosome and chromatid aberrations in the first mitotic division stratified to cancer reports in the immediate family and then to the gender and cancer diagnosis

CiF	G	CD	n	Age ±SD	AbC ±SD	CTA_{1F} ±SD	CTA_{2F} ±SD	CTA ±SD	CSA ±SD	SCE ±SD	PRI ±SD
0	all	All	73	**59.2**	**50.2**	**3.08**	**73.7**	**69.9**	**37.7**	**4.45**	**2.37**
				11.70	10.16	11.05	22.06	19.24	12.22	1.09	.40
1	all	All	5	**56.8**	**54.3**	**3.35**	**78.5**	**73.3**	**29.8**	**5.19**	**2.41**
				6.83	6.89	2.04	11.83	13.20	9.12	.23	.61
0	all	0	41	**56.9**	**49.6**	**3.18**	**73.0**	**69.1**	**37.0**	**4.57**	**2.35**
				13.30	10.72	2.86	21.09	19.99	12.46	1.27	.31
		1	32	**62.0**	**51.1**	**2.92**	**74.8**	**71.2**	**38.7**	**4.34**	**2.38**
				8.63	9.41	2.64	23.77	18.29	10.54	1.01	.40
1	all	0	nd	nd	nd	nd	nd	nd	nd	Nd	Nd
		1	5	**56.8**	**54.3**	**3.35**	**78.5**	**73.3**	**29.8**	**5.19**	**2.41**
				6.83	6.89	2.04	11.83	13.20	9.12	.23	.61
1	M	1	2	**64.0**	**48.4**	4.65	**66.1**	**59.5**	**29.8**	**4.50**	**2.34**
					2.63	1.32	1.32	.00	7.89		
	F	1	3	**52.0**	**58.3**	**2.48**	**86.8**	**82.5**	**29.8**	**5.19**	**2.41**
				2.64	5.68	2.15	4.68	5.68	11.62	.22	.60
0	M	0	19	**58.5**	**47.3**	**3.55**	**70.2**	**65.7**	**35.0**	**4.07**	**2.27**
				13.63	12.03	3.39	24.40	22.66	13.66	1.11	.36
	M	1	18	**62.6**	**50.0**	**2.58**	**72.6**	**70.1**	**38.7**	**3.72**	**2.43**
				7.24	9.95	2.20	22.84	22.50	11.78	.69	.30
	F	0	22	**55.5**	**51.6**	**2.86**	**75.5**	**72.1**	**38.7**	**5.11**	**2.49**
				13.17	9.28	2.36	17.97	17.31	11.44	.99	.16
	F	1	14	**61.4**	**52.6**	**3.38**	**77.6**	**72.6**	**38.7**	**4.90**	**2.33**
				10.40	8.85	3.20	14.07	11.80	12.66	.94	.49

Abbreviations as in the Table 12.1. (Differences statistically insignificant after grouping variables by CiF factor withnonparametric tests (Mann-Whitney U, Wilcoxon W)

This subgroup had the highest level of chromatid type damage, including highest level of SCE. The remaining patients might be considered as group with lower than others probability of hereditary predisposition to cancer. After stratifications the subgroup of cancer patients, who had cancer reports in their families according to gender, a visibly lower age of expression of the disease was seen in females patients and much higher than in males level of chromatid type of the damage (except of frequency of single chromatid fragments).

When, the rest of the group (without documented cancer in family) was stratified according to gender and finally to cancer diagnosis, the results of the rate of aberrant cells (AbC) and chromatid type of aberrations (CTA_{2F}, CTA) were still visibly higher for CCP than for control group. Ages of cancer expression in that group is higher than average age of control group (both for females and males).

Table 12.3. Mean values of cellular capacities in groups of colorectal cancer patients (CCP = 1) and controls (CCP = 0) evaluated from DNA repair competence assay before and after stratification according to the gender and cancer diagnosis

G	CD	n	T-DNA$_{X-rays}$ ±SD	TM$_{X-rays}$ ±SD	TL$_{X-rays}$ ±SD	RE$_{T-DNA}$ ±SD	RE$_{TL}$ ±SD	RE$_{TM}$ ±SD
All	0	35	30.06	35.34	66.13	92.10	79.09	94.64
			6.93	11.80	22.24	22.29	34.26	23.78
	1	26	27.19	31.38	58.85	86.70	70.63	88.46
			8.04	14.87	26.42	25.95	29.13	26.74
M	0	18	30.42	35.50	69.36	92.35	78.98	95.30
			7.12	11.61	19.23	22.00	31.62	22.42
	1	15	28.70	34.35	64.70	91.24	78.42	93.91
			8.78	16.76	28.40	27.40	33.14	28.30
F	0	17	29.68	35.17	62.70	91.83	79.20	93.94
			6.92	12.35	25.18	23.27	37.84	25.82
	1	11	25.14	27.34	50.87	80.51	60.00	81.04
			6.75	11.32	22.24	23.37	19.17	23.69

T-DNA$_{X-ray}$ – radiosensitivity of cells evaluated from immediate response to the challenging dose of X-rays, measured with SCGE technique and expressed as: T-DNA – % of DNA in a comet, TM – DNA tail moment, and TL – DNA tail length, The RE$_{T-DNA}$ express DNA damage repair efficiency (RE), defined as percent of DNA damage that was repaired during the post irradiation incubation and that was detected with the use of DNA measures (T-DNA, TM, TL – % of DNA in a comet, tail moment or tail length respectively)

Mean values of cellular capacities in groups of colorectal cancer patients (CCP = 1) and controls (CCP = 0) evaluated from DNA repair competence assay before and after gender stratification are shown in the Table 12.3. Figures 12.2 and 12.3, show examples of variability between individual susceptibility to the DNA radiation damage and efficiency of the DNA repair in the compared groups. Figures 12.2a and 12.2b show the results of the cellular radiosensitivities (DNA damage detected in cells immediately after irradiation and measured as T-DNA$_{(x-rays)}$ (Fig. 12.2a) and TM$_{(x-rays)}$ (Fig. 12.2b), respectively, for colorectal cancer patients described as the dispersion from average obtained for control group (T-DNA$_{X-rays}$ = 30.06 ± 6.93, TM$_{X-rays}$ = 35.34 ± 11.8). Figures 12.3a and 12.3b are show the results of the repair efficiency of the DNA damage induced
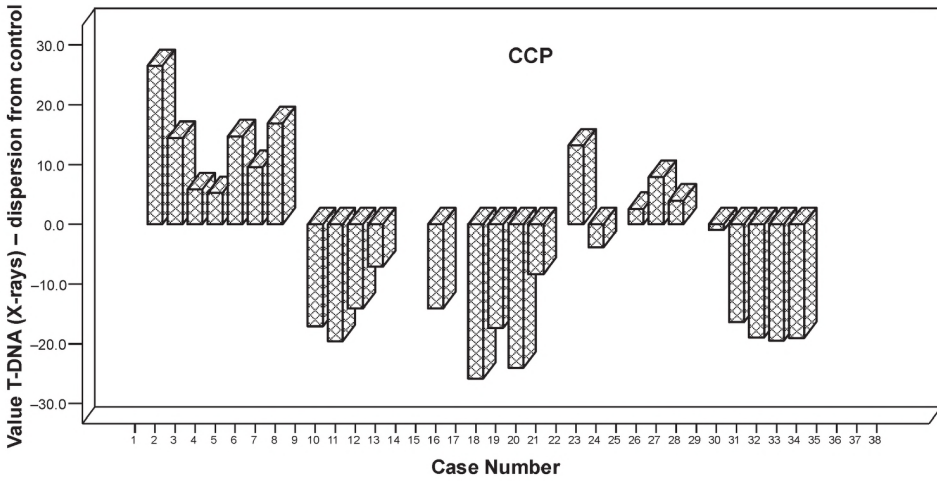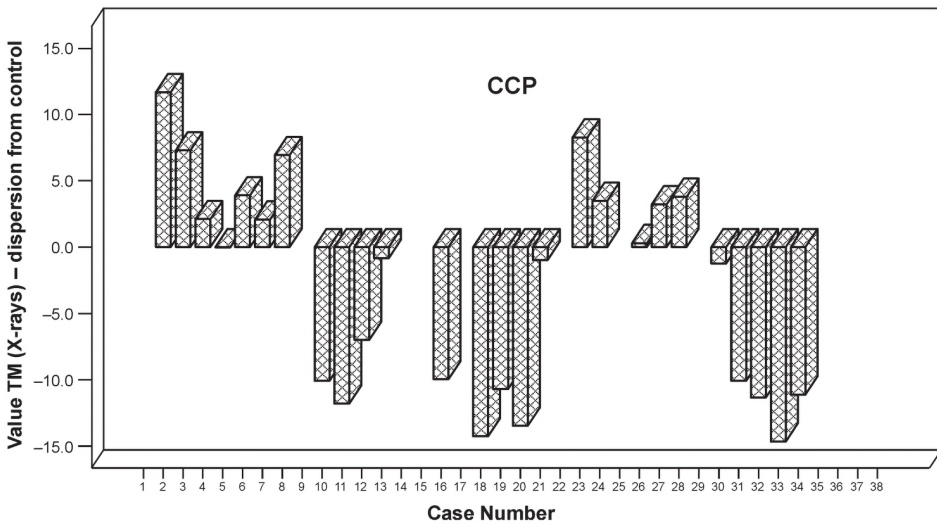
Figure 12.2a. Dispersion (from mean value obtained for the control) of radiosensitivities evaluated from DNA damage detected in response to irradiation of lymphocytes from colorectal cancer patients (based on T-DNA$_{X-rays}$ measures) described as the dispersion from average obtained for the control group (T-DNA$_{X-rays}$ = 30.06 ± 6.93)

Figure 12.2b. Dispersion (from mean value obtained for the control) of radiosensitivities evaluated from DNA damage detected in response to irradiation of lymphocytes from colorectal cancer patients (based on TM$_{X-rays}$ measures) described as the dispersion from average obtained for the control group (TM$_{X-rays}$ = 35.34 ± 11.80)

by radiation for the colorectal cancer patients, evaluated for the T-DNA measures (Fig. 12.3a) and TM (Fig. 12.3b), respectively, again described as the dispersion from average obtained for the controls (average RE$_{T-DNA}$ = 92.10 ± 22.29, and RE$_{TM}$ = 94.64 ± 23.78). In both groups of results, variability between individuals is rather high, and this affects a statistical power. Although, none of the differences between the results shown in the

Table 12.3, that might differentiate either DNA sensitivities to challenging treatment or DNA repair efficiency are statistically significant, though, overall for all DNA measures (T-DNA, TM and TL), both radiosensitivity and DNA repair efficiency is lower in colorectal patients group.
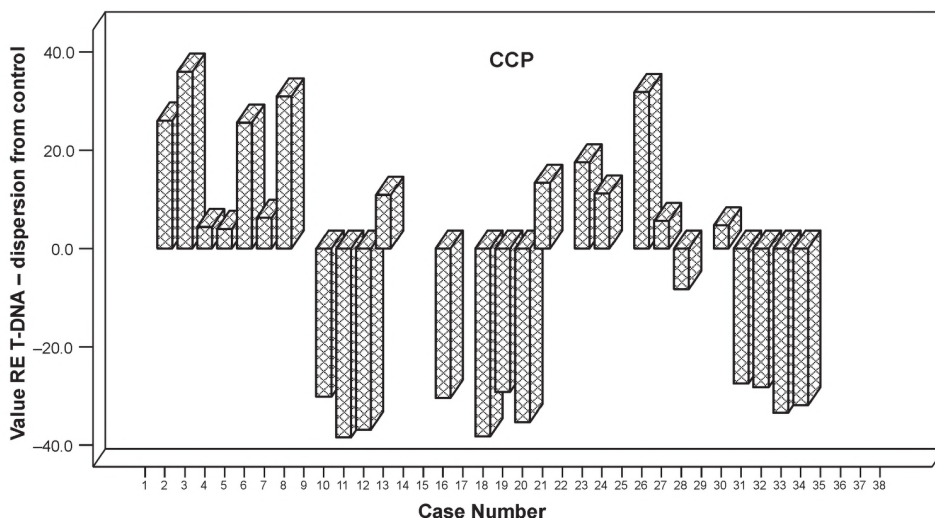

Figure 12.3a. Dispersion (from mean value of the DNA repair efficiency – RE obtained for the control group) evaluated from the DNA damage detected after post irradiation incubation in lymphocytes from colorectal cancer patients (based on T-DNA$_{rep}$ measures) described as the dispersion from average obtained for the control group (RE_{T-DNA} = 92.10 ± 22.29)
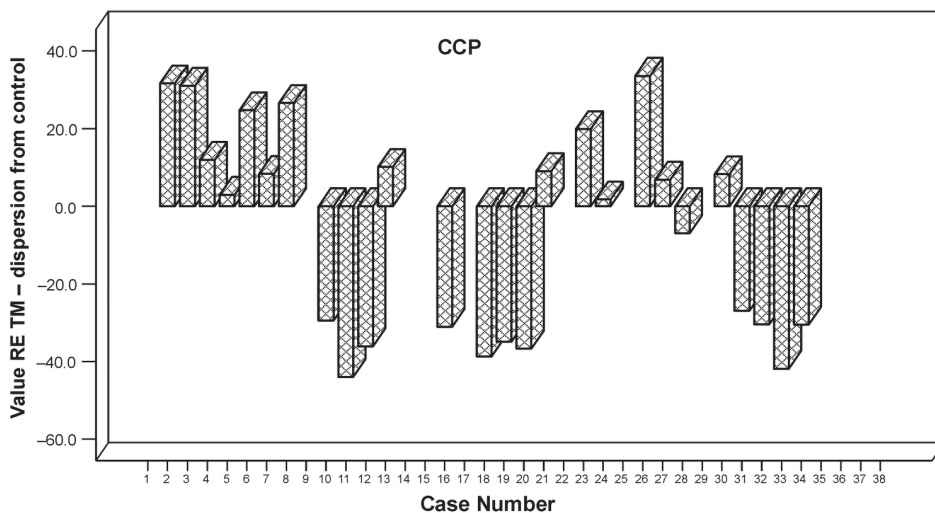

Figure 12.3b. Dispersion (from mean value of the DNA repair efficiency – RE obtained for the control group) evaluated from the DNA damage detected after post irradiation incubation in lymphocytes from colorectal cancer patients (based on T-DNA$_{rep}$ measures) described as the dispersion from average obtained for the control group (RE_{T-DNA} = 94.64 ± 23.78)

When results are stratified according to the gender, then, cellular capacities in control subgroups show comparable values between males and females. Sensitivity to challenging treatment is lower in lymphocytes of females (i.e., TLX-rays – males 66.9 vs. 62.5 females), however, in CCP groups, females expressed lower than males percentage of the repaired DNA damage (RE_{T-DNA} – CCP males 91.2% vs. 80.5% females, RE_{TM} – 93.9% males vs. 81.0% females, and RE_{TL} – 78.4% vs. 60.0%, respectively). This may correspond to the results from cytogenetics studies (Table 12.1) where in a group of CCP females, higher cytogenetic damage was observed.

Table 12.4 shows mean values of cellular capacities in groups of colorectal cancer patients (CCP = 1) and controls (CCP = 0) evaluated from DNA repair competence assay after stratification according to cancer incidence in the immediate families (CiF), then according to gender, and cancer diagnosis. Again, all factors measured with the SCGE assays are insignificantly lower in the whole group of CCP patients than in the controls.

Table 12.4. Mean values of cellular capacities in groups of colorectal cancer patients (CCP = 1) and controls (CCP = 0) evaluated from DNA repair competence assay after stratification according to cancer incidence appearing in immediate families (CiF), gender and cancer diagnosis

CiF	Gender	CD	n	T-DNA$_{X-rays}$ ±SD	TM$_{X-rays}$ ±SD	TL$_{X-rays}$ ±SD	RE$_{T-DNA}$ ±SD	RE$_{TL}$ ±SD	RE$_{TM}$ ±SD
		1	26	27.19	31.38	58.85	86.70	70.63	88.46
1	all	all	**4**	**23.65**	**27.82**	**55.84**	**76.85**	**54.63**	**77.31**
				8.09	14.42	26.42	23.98	29.80	29.61
0	all	all	**56**	**28.07**	**31.76**	**59.99**	**86.79**	**72.22**	**88.72**
				3.48	7.88	16.01	11.44	21.81	10.20
1	M	1	2	**25.20**	**30.44**	**58.91**	**75.99**	**56.22**	**76.79**
				9.82	20.62	26.62	31.57	36.99	37.04
	F	1	2	**22.11**	**25.21**	**52.77**	**77.71**	**53.05**	**77.83**
				9.51	13.08	36.71	26.93	35.87	35.46
0	M	0	17	**30.17**	**35.17**	**69.25**	**91.03**	**76.51**	**93.62**
				7.26	11.87	19.82	21.94	30.74	21.91
		1	13	**29.24**	**34.95**	**65.60**	**93.59**	**81.84**	**96.54**
				8.92	17.01	29.59	27.35	32.75	27.64
0	F	0	17	**29.68**	**35.17**	**62.71**	**91.83**	**79.20**	**93.94**
				6.92	12.35	25.18	23.27	37.84	25.82
		1	9	**25.82**	**27.81**	**50.45**	**81.13**	**61.54**	**81.76**
				6.55	11.73	21.19	24.28	16.85	23.27

Abbreviations as in the Table 12.3. (Differences statistically insignificant after grouping variables by CiF factor with nonparametric tests (Mann-Whitney U, Wilcoxon W)

The results from molecular analyses show similar pattern as observed in cytogenetics studies. CCP patients who were reporting cancers in their immediate family (CiF = 1) have shown much lower efficiency of DNA repair (in all measures T-DNA, TM, TL) than the rest of the studied group, and than the other CCP group (CiF = 0), although cellular sensitivity to radiation damage of those patients is also lower. Females, from control group (CiF = 0 and CD = 0) show similar to men sensitivity and DNA repair ef-

ficiency. Group of CCP females without cancer cases in the immediate family (CiF = 0), expressed lower DNA repair efficiency than males from the same group, in spite of slightly lower radiosensitivity.

Our results from classic cytogenetic analysis of lymphocytes from colorectal cancer patients showed higher than in controls level of chromatid type of damage, and slightly lower level of chromosome types (dicentrics, rings and acentric fragments). Cytogenetic results, to some extent, are consistent with the amount of unrepaired DNA damage induced by experimental treatment. A efficiency of DNA repair evaluated by the Comet assay does not necessarily determine fidelity of repaired DNA. Therefore, slower and less efficient DNA repair process observed in lymphocytes of colorectal cancer patients might be associated with higher amount of misrepaired DNA that would result in chromosome aberrations. This suggestion is confirmed by higher percent of induced aberrant cells and higher frequency of chromosome aberrations. Cytogenetic damage and deficiency in DNA repair show also association with smoking and sport activities (results not shown). Nevertheless, our findings need deeper molecular insight and further studies in larger populations and more homogenic, in terms of gender, smoking and other lifestyle factors, like diet, crucial for this type of dissease. According to Gonzalgo et al. (10), an alternative sign of pathway to cancer in addition to aneuploidy, loss of heterozygosity and gene mutations and the identification of molecular markers specific to early and late events in cancer progression is critical for the development of improved detection and prognostic strategies (7). Low statistical power of differences between CCP and control, can also be associated to recent findings by Delongchamps N.B. et al. (19) who have shown that benign prostatic hyperplasia but not prostate cancer was directly associated with the presence of chronic inflammation (12). It is possible that lymphocytes from CCP patients are stimulated to show strong abilities to repair DNA damage, however, cells from patients suffering with temporary infection also are less capable, because of presence of competing chronic inflammation process in the same conditions.

The results obtained from molecular and cytogenetic studies should confirm that evaluation of cellular sensitivity and repair competence could have an important value for pre-clinical studies, and that cellular DNA repair efficiency evaluated by SCGE assay may identify patients more sensitive or resistant to therapeutic treatment. The repair competence assay can provide such information during one day from collection of the biological sample. Our suggestion is important from clinical point of view, because classic cytogenetic biomarkers, although excellent and reliable, are extremely time consuming. Further and deeper studies are needed to improve statistics and fully understand mechanisms involved in various associations between molecular and cellular responses expressed in our studies.

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