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INFLUENCE OF CATALASE ON THE RADIOSENSITIVITY OF FANCONI ANEMIA LYMPHOCYTES *IN VITRO*

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Abstract — Fanconi anemia (FA) is a genetic disease characterized by progressive pancytopenia and cancer susceptibility. The clinical and cellular phenotypes of Fanconi anemia are associated with a set of redox abnormalities, indicating that FA is an oxidative stress-related disorder. Fanconi anemia cells are highly sensitive to DNA clastogen agents, but their response to ionizing radiation is still unclear. The aim of this study was to evaluate the in vitro radiosensitivity of Fanconi anemia homozygotes and heterozygotes, and to assess the contribution of catalase and superoxide dismutase (SOD) to the overall radiobiological response of the cells. The incidence of radiation-induced lymphocyte micronuclei was used as the indicator of radiation sensitivity in vitro, whereas the activity of antioxidant enzymes was determined in erythrocytes. Patients with FA exhibited a two-fold decrease in catalase activity, accompanied by lowered activity of SOD, and increased incidence of baseline micronuclei. In the entire group of patients (with one exception), a reduced yield of radiation-induced micronuclei in lymphocytes was observed, and this was categorized as a radioresistant response. A mild radioresistant in vitro response was also observed in carrier-mothers, accompanied by reduced activity of catalase. The radiosensitivity of carrier-fathers was normal. The results of this study suggest that reduced activity of catalase is an important contributor to the radiobiological response of cells.

Key words: Fanconi anemia, catalase, superoxide dismutase, radiosensitivity *in vitro*

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INTRODUCTION

Fanconi anemia (FA) is a genetically heterogeneous disease characterized by bone marrow failure, congenital abnormalities, and a significant predisposition toward malignancies, particularly acute myeloid leukemia (Okuyama and Mishina, 1987; Alter, 1996). Typically, FA patients develop bone marrow failure leading to aplastic anemia during the first decade of life, and at least 20% of them develop malignancies (Jacquemont and Taniguchi, 2007). The cellular defect responsible for FA is caused by mutation of at least 13 genes and accordingly is subtyped by 13 complementation groups abbreviated from A to N. Fanconi anemia proteins play a central role in coordination of DNA repair, cell cycle, and apoptosis pathways in response to DNA damage. With the exception of FANCD2 and BRCA2, most of the Fanconi anemia proteins are thought to form large multiprotein complexes, cytoplasmatic and nuclear. Both complexes of FANCA, C, G, E, and F proteins exist *in vivo*. Nuclear complexes are dominant, and patient-derived inactivating mutations of FANCA, FANCC, FANCE, and FANCG proteins reduce assembly, stability, or nuclear translocation of the multi-subunit Fanconi anemia protein complex. The body of evidence in FA research indicates that Fanconi anemia is an oxidative stress-related disease. Patients with FA are defective in repair of oxidative DNA damages, indicating a direct link between reactive oxygen species (ROS) formation, oxidative DNA damage, and chromosomal breakages (Joenje et al., 1981; Dallapiccola et al., 1985; Schindler and Hoehn, 1988; Pagano and Korkina, 2000). One of the main features of Fanconi ane-

mia cells is an elevated incidence of spontaneous chromosomal aberrations, which can be further triggered by clastogenic agents such as mitomycin C (MMC) and diepoxybutane (DEB) (Sasaki and Tonomura, 1973). Nowadays, the DEB-test serves as the most reliable *in vitro* method for verification of the FA cellular phenotype.

Fanconi cells are highly sensitive to DNA cross-linking agents, but their response to ionizing radiation is still unclear. Fanconi anemia cells (complementation groups FA-A, FA-C, and FA-F) show defects in rejoining double-strand breaks, but no difference in the radiosensitivity of normal and FA cells has been reported at the cellular level (Sasaki and Tonomura, 1973; Natarajan et al., 1984; Casado et al., 2005). Fanconi anemia cells also show an increase in the baseline level of micronuclei compared with normal cells, but not in the yield of micronuclei induced by H_2O_2 (Zunino et al., 2001). A number of other reports claim to have shown that FA cells are radiosensitive, and these claims are supported by clinical observations of irradiated FA patients after preconditioning for bone marrow transplantation (Gluckman et al., 1983; Gluckman, 1990). Disparity between clinical and cellular radiosensitivity has also been observed. Marcou et al. (2001) reported a severe clinical radiosensitivity in a single patient who displayed normal radiosensitivity *in vitro* as determined by the colony survival assay.

Our previous investigation of intrinsic radiosensitivity in a healthy population showed marked individual variability in radiation response and the crucial role of cellular antioxidant enzymes in this response (Joksic et al., 1999, 2000; Pajovic et al., 2000). This observation prompted us to investigate the influence of the antioxidative status of FA patients (homozygotes) and carriers (heterozygotes) on cellular radiosensitivity. The study considered a total of nine families and represents the first study of Fanconi anemia families in the Western Balkan region.

SUBJECTS AND METHODS

Subjects

A total of nine families, whose children (six girls and three boys, aged 9 years on average) were clinically diagnosed with aplastic anemia, were investigated. Five of the subjects displayed typical cellular FA phenotypes (established through the DEB-test), whereas the other four children, who had clinical manifestations of aplastic anemia but normal DEB sensitivity, were classified as non-FA patients. Peripheral blood samples from all individuals were collected by venipuncture in heparinized vacutainers in accordance with current Health and Ethical Regulations in Serbia (2005). The incidence of spontaneously occurring chromosomal aberrations, baseline levels of micronuclei (MN), radiosensitivity, and the activities of catalase and superoxide dismutase were examined.

The incidence of radiation-induced lymphocyte micronuclei was used as the indicator of radiation sensitivity *in vitro*, whereas the activity of antioxidant enzymes was determined in erythrocytes. Four of the five FA patients belong to complementation group A, whereas only one case belongs to the complementation group FANCD1 (BRCA2 mutation was found).

Chromosome aberration analysis

For scoring chromosomal aberrations, standard methods as described in IAEA (2001) were used: aliquots of heparinized whole blood (0.5 mL) were set up in cultures containing PBmax-karyotyping medium (Invitrogen-Gibco, Paisley, UK). Cells were harvested 48 h after initiation of the cultures with addition of colchicine (Sigma-Aldrich, Munich, Germany) during the last 3 h (final concentration of 2.5 μg/mL). After staining, a hundred well-spread and complete first division metaphases per subject were analyzed for chromosome and chromatid aberrations according to the "International System for Human Cytogenetic Nomenclature" (ISCN, 2005).

Irradiation

Two hours after blood collection, an aliquot of heparinized whole blood from each subject was poured into a sterile plastic test-tube positioned in a 15 x 15 cm Plexiglas container and irradiated using a ^{60}Co γ-ray source. The radiation dose employed was 2 Gy, the dose-rate was 0.45 Gy/min, dimensions of the radiation field were 20 x 20 cm, and the distance from

the source was 74 cm. Blood samples were irradiated at room temperature.

Micronucleus assay

Baseline levels of micronuclei and radiosensitivity were estimated employing the cytokinesis block micronucleus (CB-MN) method of Fenech et al. (1993). A minimum of 1000 binucleated cells were scored with an AxioImager A1 microscope (Carl Zeiss, Jena, Germany) using a magnification of 400x or 1000x when necessary.

Catalase assay

Catalase activity was measured in hemolyzates using the method of Aebi (1974) by following the catalytic reduction of hydrogen peroxide. Decomposition of the substrate H_2O_2 was measured using a Perkin Elmer Lambda 25 Spectrophotometer (Perkin Elmer Instruments, Norwalk, CT, USA) at 240 nm. Activity was expressed as K – the rate constant of the first-order reaction per minute per mg protein ($K/min^{-1} \cdot mg \cdot protein^{-1}$). Protein concentration was determined by the method of Lowry et al. (1951).

Superoxide dismutase assay

Activity of SOD was measured in hemolyzates using the Oxis Bioxytech® sod-525™ Assay (Oxis International, Inc., Portland, OR, USA). The method is based on the SOD-mediated increase in the rate of autoxidation of reagent 1 (5,6,6a,11b-tetrahydro-3,9,10-trihydroxybenzo[c]fluorene) in an aqueous alkaline solution, yielding a chromophore with maximum absorbance at 525 nm. One SOD-525 activity unit is defined as the activity that doubles the autoxidation rate of the control blank. The assay was performed using a Perkin Elmer Lambda 25 spectrophotometer (Perkin Elmer Instruments, Norwalk, CT, USA). The specific enzyme activity of SOD was expressed as units per mg of protein (U/mg of protein). Protein concentration was determined by the method of Lowry et al. (1951).

Statistical Analysis

Statistical analysis was carried out with the statistical software package Statistica 6.0 for MS Windows using Spearman rank order correlations (R) and Student's t-test. Values of P at the level of a 95% confidence limit were considered significant.

RESULTS

The incidence of spontaneously occurring chromosomal aberrations, micronuclei, and radiosensitivity for both subgroups (FA and non-FA families) are presented in Table 1; the activities of antioxidant enzymes (catalase and superoxide dismutase) are presented in Table 2 and Fig. 1.

The analysis of chromosomal aberrations considered chromatid- and chromosome-type breaks, acentric fragments, symmetrical and asymmetrical chromosomes, and chromatid exchanges. Chromatid- and chromosome-type breaks and acentric fragments were considered to be one-break events, while dicentrics, rings and radial figures were counted as two breaks (Auerbach et al., 1989; Wegner and Stumm, 1999).

The incidence of spontaneously occurring chromosomal aberrations in FA patients was almost 4.5-fold higher than that in non-FA individuals.

The baseline level of micronuclei in the entire group of children was 11.69 ± 6.82 (14.24 ± 7.61 in FA children, 8.52 ± 4.77 in non-FA children). The higher incidence of baseline micronulei in FA patients was accompanied by a 1.75-fold higher percentage of micronucleated BN cells. A positive, statistically significant correlation between baseline levels of micronuclei and incidence of chromosomal aberrations was observed in FA patients ($p < 0.05$).

Fanconi anemia mothers showed a 73% higher baseline level of micronuclei compared to non-FA mothers, whereas no differences in baseline level of micronuclei were observed between FA and non-FA fathers.

Similar results were obtained comparing the percentage of micronucleated cells in FA- and non-FA mothers (1.57-fold increase in FA carriers), whereas no differences between FA- and non-FA fathers were found.

The yield of radiation-induced micronuclei (ra-

Table 1. Incidences of chromosomal aberrations, baseline levels of micronuclei, and radiosensitivity in FA- and non-FA families. Abbreviations: CA - incidence of chromosomal aberrations (breaks per cell); MN - baseline level of micronuclei (MN/1000 BN cells); MNri - incidence of radiation-induced micronuclei (MN/1000 BN cells); % of mBN cells - percentage of micronucleated binucleated cells..

FA families	**CA**	**MN**	**% of mBN cells**	**MNri**	**% of mBN cells**
Patient					
1	0.071	21.32	1.84	150.20	14.99
2	0.036	8.50	0.66	71.47	7.54
3	0.230	21.41	2.14	360.51	31.30
4	0.130	15.51	1.55	53.39	6.69
5	0.030	4.45	0.44	96.31	8.90
Mean ± SD	0.099 ± 0.083	14.24 ± 7.61	1.33 ± 0.74	146.38 ± 125.13	13.88 ± 10.26
Mother					
1	0.000	21.49	2.15	118.43	12.14
2	0.000	5.40	0.54	174.29	15.63
3	0.120	41.10	3.20	180.78	18.44
4	0.000	10.18	0.68	136.79	13.48
5	0.000	8.25	0.74	157.65	14.93
Mean ± SD	0.002 ± 0.005	17.28 ± 14.65	1.46 ± 1.17	153.59 ± 25.99	14.92 ± 2.38
Father					
1	0.050	24.04	2.02	146.46	15.23
2	0.010	12.40	1.24	213.45	18.43
3	0.010	14.54	1.35	215.32	19.35
4	0.000	14.86	1.39	111.62	10.41
5	0.000	11.61	0.98	164.03	15.30
Mean ± SD	0.014 ± 0.021	15.49 ± 4.98	1.40 ± 0.38	170.18 ± 44.55	15.74 ± 3.50
Non-FA families	CA	MN	% of mBN cells	MNri	% of mBN cells
Patient					
6	0.030	9.77	0.98	58.88	6.59
7	0.020	8.96	0.90	146.23	13.54
8	0.020	1.97	0.20	79.70	7.73
9	0.018	13.39	0.98	86.98	9.12
Mean ± SD	0.022 ± 0.005	8.52 ± 4.77	0.76 ± 0.38	92.95 ± 37.46	9.25 ± 3.04
Mother					
6	0.020	6.33	0.54	120.64	11.65
7	0.000	11.48	1.15	184.06	16.83
8	0.020	9.41	0.94	198.86	17.82
9	0.000	12.75	1.08	202.83	17.62
Mean ± SD	0.010 ± 0.011	9.99 ± 2.80	0.93 ± 0.27	176.60 ± 38.17	15.98 ± 2.92
Father					
6	0.040	31.70	2.50	304.84	25.07
7	0.040	3.92	0.39	140.17	11.83
8	0.000	17.74	1.40	144.97	14.58
9	0.000	14.06	1.31	252.73	22.85
Mean ± SD	0.020 ± 0.023	16.86 ± 11.49	1.40 ± 0.86	210.68 ± 81.49	18.58 ± 6.37

Table 2. Activities of catalase and superoxide dismutase (SOD) in FA- and non-FA families..

FA families	Activity of catalase (min^{-1} · mg of $protein^{-1}$)	Activity of SOD (U/mg of protein)	Non-FA families	Activity of catalase (min^{-1} · mg of $protein^{-1}$)	Activity of SOD (U/mg of protein)
Patient			**Patient**		
1	8.26	31.13	**6**	18.36	26.37
2	8.51	31.57	**7**	31.33	36.92
3	11.26	29.67	**8**	15.02	34.36
4	9.96	31.23	**9**	21.56	33.46
5	14.42	21.37			
Mean ± SD	10.48 ± 2.51	28.99 ± 4.32	Mean ± SD	21.57 ± 7.03	33.45 ± 5.45
Mother			**Mother**		
1	8.11	44.00	**6**	10.40	28.44
2	4.78	25.63	**7**	27.17	40.81
3	4.57	19.14	**8**	11.09	38.96
4	12.21	46.25	**9**	16.23	36.08
5	11.39	26.35			
Mean ± SD	8.21 ± 3.58	32.27 ± 12.09	Mean ± SD	16.22 ± 7.75	36.07 ± 5.44
Father			**Father**		
1	7.09	34.88	**6**	11.37	29.60
2	8.56	45.15	**7**	22.15	52.50
3	8.96	44.60	**8**	8.39	49.91
4	11.02	40.39	**9**	13.98	44.01
5	19.40	36.87			
Mean ± SD	11.00 ± 4.90	40.38 ± 4.56	Mean ± SD	13.97±5.91	44.00 ± 10.24

diosensitivity) was 122.63 ± 95.65 in the entire group of patients, 146.38 ± 125.13 in FA-children, and 92.95 ± 37.46 in non-FA children, whereas incidences of radiation-induced micronuclei in FA parents were slightly lower (161.88 ± 35.48) compared to non-FA parents (193.64 ± 61.66). Higher incidence of radiation-induced micronuclei in the FA group was accompanied by a 1.5-fold higher percentage of BN cells carrying micronuclei compared to non-FA.

A statistically significant difference of catalase activity was found in FA children when compared to non-FA children, showing about two-fold decrease ($p < 0.05$). Catalase activity in carrier FA mothers was almost two times lower than that observed in non-FA mothers, whereas FA fathers displayed only slightly reduced activity of catalase when compared to non-FA fathers (Fig. 1).

Activity of SOD in FA children was 13% lower than that observed in non-FA children, without statistical significance. Lack of statistical significance was also found comparing SOD activity between FA carriers and non-FA parents.

DISCUSSION

The aim of this study was to evaluate the contribution of antioxidant enzyme activities to the overall radiobiological response of FA lymphocytes. Radiosensitivity of FA cells, measured by micronuclei formation, significantly differed from that of normal cells. In our previously conducted study ($n = 82$), cluster analysis for yields of radiation-induced micronuclei defined normal sensitivity and radiosensitive and radioresistant cellular responses (Joksic et al., 1999). Corresponding yields of micronuclei were 200 ± 29, 322 ± 31, and 136 ± 23, respectively. In the group of five FA patients, three of them (patients 2, 4, and 5) showed strong radioresistant

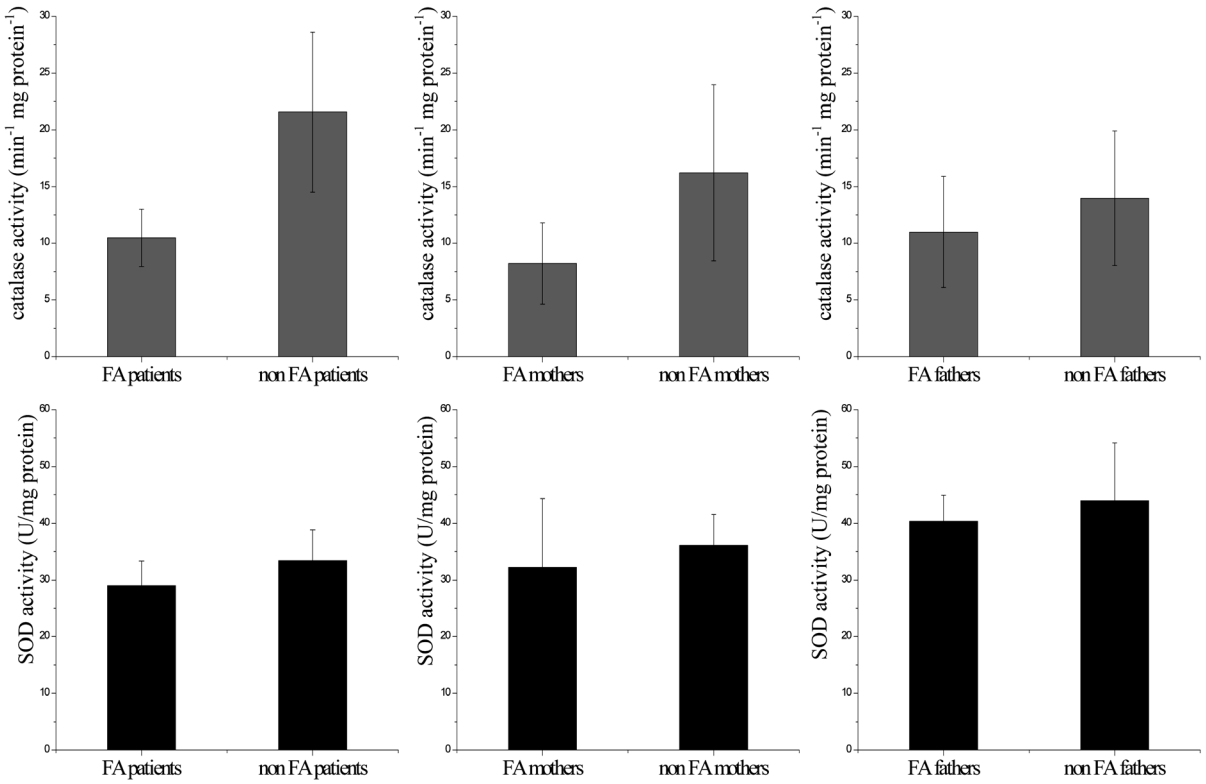

Fig 1. Activities of catalase and superoxide dismutase (SOD) in FA- and non-FA families (means ± SD)..

responses, one (patient 1-BRCA2) showed a weak radioresistant response, and only one (patient 3) showed a radiosensitive response following *in vitro* irradiation. Fanconi anemia mothers also displayed a weak radioresistant response, while FA fathers showed normal radiosensitivity. In the group of four non-FA patients, three of them (patients 6, 8, and 9) showed strong radioresistant responses and one (patient 7) showed a weak radioresistant response following *in vitro* irradiation. Non-FA mothers as well as non-FA fathers displayed normal sensitivity to ionizing radiation *in vitro.*

A radioresistant response of FA lymphocytes (with one exception) following *in vitro* irradiation and reduced activity of catalase were found in the present study. It is known that catalase is a key enzyme of the cell antioxidative defense, since its catalytic removal of H_2O_2 prevents hydroxyl radical formation. Consequences of catalase deficiency include formation of DNA base adducts such as 8-hydroxy-2'-deoxyguanosine (8-OHdG) and increased production of H_2O_2. It has been reported that large amounts of H_2O_2 induce apoptosis (Zunino et al., 2001; Bai and Cederbaum, 2003; Saadatzadeh et al., 2004). The results of our study showed that FA patients exhibited a two-fold decrease in catalase activity when compared to non-FA patients. Their parents (heterozygous carriers), especially the mothers, also displayed reduced catalase activity, associated with resistant responses to *in vitro* radiation. We hypothesize that the reduced yield of radiation-induced lymphocyte micronuclei *in vitro* was a consequence of catalase deficiency, which in turn leads to overproduction of H_2O_2 and an increased portion of cells undergoing apoptosis. Apoptosis may be secondarily triggered by oxidative stress or DNA damage. Additional production of reactive oxygen species by irradiation generates

intermediates that are capable of inducing apoptosis (Marnett, 2000). It is also important to mention that sex hormones are recognized as modulators of oxidative stress (Tam et al., 2003). Gender- and age-associated differences of oxidative stress parameters in FA patients and carriers have been reported before (Pagano et al., 2004). Thus, it is not surprising that androgen administration has beneficial effects for most FA patients.

Compared to non-FA patients, FA patients displayed a higher incidence of radiation-induced micronuclei, a higher percentage of micronucleated cells, and a two-fold decrease in catalase activity. However, when compared with responses to *in vitro* radiation in healthy members of the population, this response belongs to the radioresistant category. A weakened antioxidative defense system most likely enables overproduction of 8-OHdG and other oxygen radicals, which consequently increase the level of cellular "free" iron ions that catalyze Fenton reactions, enhancing radiotoxicity (Korkina et al., 1992). Decreased catalase activity had been previously reported in FA patients belonging to complementation group A (Takeuchi and Marimoto, 1993). Increased formation of 8-OHdG was also detected in FA patients, as well as in carriers (Degan et al., 1995). It is known that 8-OHdG is the most mutagenic among various oxidative DNA lesions. If left unrepaired, the outcome of this lesion is a G→T transversion, a mutation frequently found in tumor-relevant genes (Loft and Poulsen, 1996). In summary, decreased activity of catalase allows overproduction of oxidative damages in DNA, leading to increased incidence of chromosomal aberrations and micronuclei. Irradiation further damages cells: overdamaged cells might die before entering mitosis, and the surviving fraction of cells might carry multiple injuries.

In FA patient number 3, where a radiosensitive *in vitro* response was observed, further investigations are needed to examine checkpoints. A high percentage of micronucleated binucleate cells as well as increased yield of radiation-induced micronuclei were likely due to checkpoint failure, and cells carrying unrepaired or missrepaired DNA damages then reached mitosis, thus producing micronuclei.

The activity of SOD in FA patients was slightly (13%) reduced compared to non-FA patients and 17% lower than in healthy children (Stojiljkovic et al., 2007). Heterozygotes in the entire group displayed similar values of SOD activity to those in the general population. In regard to the activity of SOD, a key enzyme that detoxifies superoxide anions, the literature provides inconsistent results: significantly reduced in erythrocytes (Joenje et al., 1978, 1979; Mavelli et al., 1982; Yoshimitsu et al., 1984) and normal in FA fibroblasts and white blood cells (Gile et al., 1987; Ruppitsch et al., 1997; Pagano and Korkina, 2000).

In the present study, decreased activity of SOD was accompanied by significantly lowered activity of catalase, indicating a weak antioxidative defense system in FA hematopoietic progenitor cells. It follows that oxidative damages to DNA might overwhelm the cellular repair capacity and lead to enhanced formation of chromosomal aberrations.

The incidence of spontaneous occurrence of chromosomal aberrations in FA patients was 4.5-fold higher than that observed in non-FA patients, and the baseline level of micronuclei in FA patients was 67% higher compared to non-FA and 2.5-fold higher than in healthy children (Neri et al., 2005).

Increased baseline levels of micronuclei in carriers were also found in the current study: FA mothers showed a 73% higher baseline level of micronuclei compared to non-FA and a two-fold increase compared to the healthy population.

A radioresistant response in FA patients (with one exception) following *in vitro* irradiation was demonstrated in the present study. These results are in agreement with other studies in which the authors did not find an increased sensitivity to ionizing radiation in FA patients (Darroudi et al., 1985; Duckworth-Rysiecki and Taylor, 1985; Barquinero et al., 2001). Micronucleus formation is one of the clastogenic effects of radiation. Radioresistant cells, which display a reduced yield of radiation-induced micronuclei, usually lack mitochondrial (mt) DNA and are less likely to undergo apoptosis (Yoshioka et al., 2004). A reduction in mitochondrial gene

expression, known to accompany mtDNA damage, can limit availability of key components of the electron transport chain, which in turn could re-route the flow of electrons and thereby augment mitochondrial oxidant production. It is known that mtDNA in cells plays major roles in ATP production, generation of ROS, and regulation of apoptosis. Owing to this, the mitochondrial genome is considerably more sensitive to oxidative damage than nuclear DNA. Cells lacking mtDNA are more radioresistant. It can therefore be concluded that serious mitochrondrial damages exist in cells of the entire group of our children, which should be examined further. Heterozygous individuals from FA families have not been extensively examined, as they are free of major clinical symptoms. It is therefore worth noting that in our study FA mothers also displayed a weak radioresistant response, while FA fathers showed normal sensitivity following *in vitro* irradiation. The radioresistant response was accompanied by a low percentage of micronucleated binucleated cells (half the normal level) and reduced activity of catalase. To our best knowledge, this is the first report regarding mother-carriers. We hypothesize that mtDNA damages exist in the maternal genotype, are inherited in the offspring, and contribute to progression of the disease. Radiosensitivity testing possibly could serve as a rapid screening test for identification of heterozygotes.

Various FA proteins are reported to be involved in the apoptotic pathway, which could explain the defects in hematopoiesis that are common to all FA patients, regardless of their complementation group (Bogliolo et al., 2002).

Studies of clinical and cellular radiosensitivity of FA cells have reported variable results with no firm conclusions. Micronuclei are clearly an important manifestation of DNA damage. The behavior of cells after irradiation can certainly provide additional information to clinicians and assist in diagnosis of the disease and chemoprevention of its progression. Further investigations are needed to determine the full significance of the radioresistance phenomenon and evaluate whether it is a prognostic factor associated with progression of the disease.

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УТИЦАЈ КАТАЛАЗА НА РАДИООСЕТЉИВОСТ ЛИМФОЦИТА *IN VITRO* КОД ПАЦИЈЕНАТА ОБОЛЕЛИХ ОД ФАНКОНИЈЕВЕ АНЕМИЈЕ

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Фанконијева анемија (ФА) је генетичко обољење карактерисано прогресивном панцитопенијом и предиспозицијом ка малигнитетима. Асоцирано је са низом абнормалности у редокс процесима у ћелији што га чини и болешћу оксидативног стреса. ФА ћелије су хиперосетљиве на ДНК кластогене агенсе, док су подаци о њиховој осетљивости на јонизујуће зрачење контрадикторни. Циљ ове студије је испитивање *in vitro* радиоосетљивости ФА хомозигота и хетерозигота и утврђивање утицаја активности каталазе и супероксид дисмутазе (СОД) на укупан радиобиолошки одговор ћелија. ФА пацијенти испољавају двоструко снижење активности каталаза, снижену активност СОД и повећани базални ниво микронуклеуса. Радиорезистентан одговор на јонизујуће зрачење (са једним изузетком) утврђен је у целој групи пацијената. Благи радиорезистентни *in vitro* одговор на јонизујуће зрачење као и снижена вредност каталаза утврђен је и код носиоца-мајки. Носиоци-очеви су испољили нормалну радиоосетљивост. Резултати ове студије показују да снижена вредност каталаза значајно доприноси укупном радиобиолошком одговору ћелија.