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The Influence of Individual Genome Sensitivity in DNA Damage Repair Assessment in Chronic Professional Exposure to Low Doses of Ionizing Radiation

Mirta Milić[1], Ružica Rozgaj[1], Vilena Kašuba[1], Ana Marija Jazbec[2],
Patrizia Hrelia[3] and Sabrina Angelini[3]
[1]Institute for Medical Research and Occupational Health, Mutagenesis Unit, Zagreb
[2]Faculty of Forestry, University of Zagreb, Zagreb,
[3]Department of Pharmacology,University of Bologna, Bologna,
[1,2]Croatia
[3]Italy

1. Introduction

During their working and living activities, humans are constantly exposed to different environmental genotoxic agents. Biological consequences of the exposure are accumulation of different mutations and DNA damage that can lead to disruption of genetic material. Numerous endogenous genotoxic agents can also cause DNA damage. Loss of normal cell function can cause cell death or can result in different health disorders, including teratogenic and cancerogenic effects (Jeggo & Lavin, 2009). There is an increasing concern about mutagenic and cancerogenic effects of genotoxic agents and their influence on individuals who are exposed to them by accident, or by living/working lifestyle (Au, 1991; Carrano & Natarajan, 1988; Kassie et al., 2000).

Exposure to ionising radiation, as a well known genotoxic agents (Balasem & Ali, 1991; Erexon et al., 1991; Fenech et al., 1990; He et al., 2000; Jeggo & Lavin, 2009), can leed to different DNA damage, such as oxidative damage, base and sugar modification in DNA, apurinic/apirimidinic places, single/double strand chromosomal breaks, adduct creation, inter/intra DNA cross linking and other types of damage (Hall & Giaccia, 2006). Numerous studies deal with ionising radiation exposure of individuals who are chronically professionally exposed to low doses (Andreassi, 2009; Au, 1991; Carrano & Natarajan, 1988; Kassie et al., 2000). The results showed the increase in chromosomal damage during chronically low dose exposure (Barquinero et al., 1993; Boutcher, 1985; Cardoso et al., 2001; Jha, 1991; Nowak & Jankowski, 1991), but without confirmed relationship between the received dose and the intensity of DNA damage (Bolus, 2001; Coates et al., 2004; Morgan, 2003; Mothersill et al., 2000, 2001; Seymour & Mothersill, 2000). The influence of chronically low dose exposure has been considered mutagenic and cancerogenic (Fachini et al., 2009). Although it is possible to estimate the influence of absorbed dose and the effect on the

exposed individuals, consequences of continuous low dose exposure are still a great topic of researches.

The medical stuff represents the best investigated group of professionals exposed to low levels of ionising radiation (UNSCEAR, 2000). During their work, all the employees wear dosimeters (film, TLD or electronic ones) that are analysed on monthly bases. In addition, they are under regular medical control.

Duration and the intensity of exposure to low doses of ionising radiation have significantly decreased over the last decades. The results of analysed dosimeters showed that the received doses are significantly below the regulatory threshold of 20 mSv per year, sometimes even under the dosimeters detection abilities (Kubelka et al., 2011).

Persons employed in emergency medical units and nuclear medicine workers are exposed to higher doses and show higher DNA damage than workers in diagnostic radiology departments (Sari-Minodier et al., 2007).

Andreassi et al. (2009) reported higher amount of DNA damage among interventional cardiologists when compared to clinical cardiologists. Epidemiological researches have shown the connection between the amounts of accumulated doses and the risk of tumour developing (Berrington et al., 2001; Maitre et al., 2003; Wang et al., 2002; Yoshinaga et al., 1999). Cardisi et al. (2005) reported the relationship between exposure to low doses of ionising radiation and cancer in study that involved 400 000 nuclear industry workers.

Today's general opinion is that initial event in radiation carcinogenesis is un/ missrepaired double strand break of DNA molecule, which is a major lesion that leads to developing of chromosomal abnormalities and genetic mutations (Little, 2000).

Accidental or therapeutical acute exposure to ionising radiation can cause different cytogenetic damage, including higher amount of micronuclei (small amount of chromatin in the shape of small nuclei in cytoplasm that was not divided into two new nuclei after first cell division) and chromosomal aberrations (single /double strand breaks of chromosome, inter/intra chromosomal exchange, dicentrics, acentrics, etc.). Recently, different biomarkers are used for monitoring of people occupationally exposed to chronical low doses of ionising radiation. The use of combined biomarkers can offer better assessment and health care of those individuals. With them a phenomenon of adaptive response has been observed. This response can be seen after the first adapting dose of ionising radiation is received (mostly under 10 cGy). After exposure to higher, so called challenging dose, above 100 cGy, those individuals show lower amount of DNA damage when compared to those who did not receive the first, adapting dose. There is also evidence that the amount of dose received can influence the adaptive response (Gourabi & Mozdarani, 1998). A great variability in DNA damage response to ionising radiation exposure of cell lines *in vitro* and individuals *in vivo* have been reported (Bosi & Olivieri, 1989; Shadley & Wiencke, 1989). Adaptive response has been shown also in persons after clinical, environmental or working exposure (Barquinero et al., 1993, 1995, 1996; Monsieurs et al., 2000; Padovani et al., 1995; Szumiel, 1998; Tedeschi et al., 1995, 1996). Individual differences in DNA repair genes can also influence on this response (Milić, 2010).

Molecular mechanisms of adaptive response are still not clear. It has been considered that it depends on the synthesis and/or protein expression, especially those involved in DNA repair mechanism (Boothman et al., 1989; Ikushima, 1989, 1996; Robson et al., 1999; Wolf et al., 1989, 1996; Youngblom et al., 1989). Early induction response (Okayasu et al., 2000;

Stecca & Gerber, 1998), changes in gene expression, gene transcription regulation are also related with adaptive response. Mutations in genes involved in DNA repair can cause DNA missrepair, chromosome endings fusion, fusion of unprotected telomere ends and double strand breaks after exposure to ionising radiation (Bailey et al., 2004; Bailey & Goodwin, 2004; Sasaki et al., 2002).

The accuracy of any risk assessment, especially low dose exposures, depends on both the resolution of the method, and the baseline data obtained in well-selected controls. Alkaline comet assay (single-cell gel electrophoresis, SCGE) is an easy-to-use, quick and very sensitive method for detecting primary DNA strand breaks, that is, direct DNA damage within single cells (Tice et al., 1990; Collins, 2004) and can be applied to proliferating and non proliferating cells (Kassie et al., 2000) to determine DNA damage as a result of endogenous factors, lifestyle (Hoffman & Speit, 2005), and occupational or environmental exposure (Valverde et al., 1999).

After relaxation, DNA can be seen as a comet during electrophoresis due to strand breaks (Singh, 2000). The advantages of this technique are sensitivity, reproducibility, easy to use, low expences. It is a rapid method and the amount of sample necessary for the analysis is very small. Comet assay enables analysis of any sort of cells, whether it is plant, animal or human origin, no matter are those single cells from the cell culture or from the tissue. Due to the short-time performance of this technique (it is possible to have results after few hours), it has become well accepted in investigation of different genotoxic agents both *in vitro* and *in vivo* conditions (Betti et al., 1994; Kassie et al., 2000; Kruszewski et al., 1998; McKelvey-Martin et al., 1993; Wojewodzka et al., 1998).

In this method, single cells are embedded into so called agarosis sandwich. Cytoplasm and membranic cell structures are lysed with high concentrated EDTA solution (ethylenediaminetetraacetic acid) and detergents, causing total cell DNA to be free. There are two comet assay protocols, whether alkaline or neutral denaturation is used.

Neutral version enables detection of double stranded DNA breaks (Olive et al., 1990). Under alkaline conditions, single stranded breaks, alkaline labile sites (AP, parts of DNA that can break easily in alkaline solutions), DNA-DNA and DNA protein crosslinking can be detected. Alkaline labile sites are apurinic and apirimidinic DNA bases that can break easily when exposed to alkaline conditions (Singh et al., 1988). After alkaline/neutral denaturation, the cells undergo electrophoresis. Concerning the high molecular weight of the entire DNA molecule, DNA can not pass through the agarose pores towards the anode. Only the small, damaged fragments can pass through. During electrophoresis, the velocity of those fragments that are caused by single or double chromosome breaks depends on their molecular weight. The smaller they are, the faster they get (Plappert et al., 1995). After electrophoresis, the slides are stained with fluorescent dye (usually ethidium bromide). Epifluorescent microscope is used for single cells analysing. Undamaged cells have round shape, while damaged one are similar to the shape of the comet. The mostly used parameters of comet assay are the tail length (TL), percentage of DNA in tail (TI) and the tail moment (TM). Tail length (usually expressed in micrometers) is measured from the centre of the comet head till the end of the comet tail (that is the distance of the fragments from the major DNA that have travelled through the gel during electrophoresis). It is proportional with DNA damage and with length of the fragments (Singh et al., 1988; Tice et al., 1990). TI is measured with computer programme for comet assay analysis. The amount of the

damage is estimated based on the ratio of DNA percentage in head and in tail of a comet. Some researchers prefer tail moment as the most reliable marker of DNA damage, because it combines measurements of tail length and percentage of DNA in tail (Ashby et al., 1995; Hellman et al., 1995; 1997; Mc Kelvey-Martin et al., 1998). Collins (2004) emphasizes the advantage of TI considering that the percentage of the tail DNA reflects the real DNA damage. Comet assay can also detect apoptotic and necrotic cells. Apoptotic cells show small comet head, and most of DNA is spread in tail in the shape of a cloud (Fairbairn et al., 1995; Olive, 1999). Comet assay is also a valuable technique to study the kinetics of primary DNA damage. It enables to estimate the DNA damage level immediately after the exposure, even when the exposure included very small dose in very short exposure period (Tice et al., 1990; Plappert et al., 1995). Fast repair can represent a problem in DNA damage evaluation in populations occupationally exposed to low doses of ionising radiation and therefore the development of sensitive methods is necessary for those experiments. Most of the primary DNA damage is repaired 30 minutes after the exposure to ionising radiation (Frankenberg-Schwager, 1989), and 2 hours after the exposure to dose of 2 Gy, most of the damaged DNA is totally repaired (Plappert et al., 1997).

Polymorphism by definition is expression of different phenotypes in the same species due to the change/s in genotype. They usually include loss (deletion) of small or bigger part of DNA molecule, insertion of specific number of nucleotides or repetition of di-, three-, or oligonucleotides in variant number. The number or repeating differs among individuals.

Variations in human genome are usually caused by variations in DNA sequence, that is based on the change of only one nucleotide (one from the four nucleotides; A-adenine, T-thymine, C-cytosine or G-guanosine is replaced by the other) usually known as SNP polymorphism (*single nucleotide polymorphism*). Among almost 15 million of SNPs in human genome, 50.000 to 100. 000 of them can change the function or gene expression.

The connection of the change in only one nucleotide (that happens once in every 1000nucleotides in human genome) with the complex aetiology of malignant diseases is poorly investigated (Bonassi et al., 2005). More than 7 millions of well known SNPs in human genome appear with the allelic frequency higher than 5% of the entire population (Hinds et al., 2005). More than 70% of SNPs in human population have the frequency less than 5 % and those SNPs are called rare SNPs (Shastry, 2009). The results of new experiments have shown the connection between gene polymorphisms and risk association with disease developing (Norpa, 2004), especially in polymorphisms of DNA repair genes and folic acid metabolism. Polymorphisms can lead to different gene expression (decreased or increased) and through this process can influence on cell repair mechanisms (Hung et al., 2005; Parl, 2005; Weiss et al., 2005; Kotsopoulos et al., 2007). Variations in DNA repair capacity have been also observed among healthy individuals (Setlow, 1983).

Among 130 genes involved in DNA repair mechanisms, 80 of them are carriers of more than 400 SNPs (Mohrenweiser et al., 2003). DNA damage and repair correlate with the radiation sensitivity and are important in radiation protection and radiotherapy (Ross et al., 2000). Due to individual variations, some persons have higher sensitivity when compared with general population (Berwick, 2000). It has been estimated that 10 -15 percent of healthy people have phenotype that shows decreased possibility for successful DNA damage repair (Mohrenweiser & Jones, 1998; Hu et al., 2002a). Higher risk of mutations, genome instability and malignant tumours have been observed among persons

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with decreased DNA repair capacity (Berwick & Vineis, 2000; Chen et al., 2002; Collins & Harrington, 2002; Divine et al., 2001; Hou et al., 2002; Kumar et al., 2003; Sturgis et al., 1999; Winsey et al., 2000).

The connection between ionising radiation and SNPs in gene involved in DNA repair has been described by several authors (Aka et al., 2004; Hu et al., 2001; Lunn et al., 2000; Touil et al., 2002). There is an increased interest for exploring of SNPs of genes that are part of biological response to ionising radiation and connecting them with clinical sensibility. Those SNPs could be used for an estimation of exposure to ionising radiation (Andreassen, 2005).

Determination of high risk population on the basis of genetic polymorphism could help in tumour prevention. The influence of polymorphisms can be crucial in the exposure to low doses of ionising radiation (Boffetta et al., 1999).

2. Materials and methods

This study included 126 subjects, 70 medical workers occupationally exposed to low doses of ionising radiation (gastroenterologists, cardiologists, anaesthesiologists, surgeons, radiologists, radiology technicians, nurses) of both gender (45 females, 25 men; mean age was 40 years, from 20-60 years old) and 56 individuals in control group who were not exposed to neither ionising radiation nor to chemical mutagens (14 women and men; mean age 40 years, from 23 to 60 years old) (Table 1).

	Control group	Exposed group
Subjects (No.) F/M	56 14/42	70 45/25
Age±SD (Min-Max)	40.53±10.92 (23-60)	40.27±10.8 (20-60)
Smoking, Y/N	16/40	31/39
Alcohol, Y/N	35/21	21/49
Years of exposure±SD (Min-Max)	-	12.22±8.65 (1-38)

Table 1. Characteristics of the control and exposed group considering the gender, age, years of exposure, smoking status and alcohol consumption.

The examinees were informed of the study scope and experimental details, have filled a standardised questionnaire designed to obtain relevant information on the current health status, medical history, and lifestyle, and gave their written consent, submitted and approved by the local Ethics Committee. The questionnaire included data on the exposure to possible confounding factors: smoking, alcohol consumption, use of medicines, contraceptives, severe infections, or viral diseases over the past six months, vitamin intake, recent vaccinations, presence of known inherited genetic disorders and chronic diseases, family history of cancer, exposure to diagnostic X-rays. Subjects with history of previous radio- or chemotherapy were not included. Exposed group was under regular film dosimetry and the dose received did not exceed 20mSv/year (data not shown).

Two millilitres of venous blood was stored in heparinised vacutainers for comet assay and assessment of DNA repair kinetics and stored at +4°C before further procedure. Detailed protocol is described before (Milić et al., 2010). Five millilitres of venous blood was stored in vacutainers with EDTA (ethylenediaminetetraacetic acid) at -20°C until further DNA isolation (Milić et al., 2010). Blood samples were irradiated with 60Co (Alcyon, CGR-MeV, France). The doses used were 2 and 4 Gy, with the same distance from the source (80 cm). Irradiation field was 15 x 15 cm². After irradiation, samples were kept at + 4°C to prevent the repair of the damage. Details are also described before (Milić et al., 2010).

2.1 Comet assay

Alkaline version of comet assay (Singh et al., 1988) with small modifications was used. Detailed procedure is described elsewhere (Milić, 2010; Milić et al., 2010). The slides were marked and stored at +4°C till the beginning of the irradiation. Control samples were put into cold lysis solution immediately after preparation and left there for 24 hours at +4°C (Milić, 2010). Irradiated blood gel samples were incubated at 37°C in serum free RPMI 1640 medium. Zero samples were immediately immersed into lysis solution. DNA repair kinetics was measured at 0, 15, 30, 60 and 120 minutes after exposure, and additional 24 hours for samples irradiated with the dose of 4 Gy. Specific measure points were based on the results of other researches (Singh et al., 1988; Price, 1993; Tice, 1995). The 2 Gy dose was a standard daily dose in radiotherapy, while the 4 Gy dose was chosen as a ˝challenging˝ dose for the exposed group. After the repair, slides were vertically placed into cold lysis solution at 4°C, overnight. Protein denaturation and DNA unwinding were done at 4°C in denaturation buffer (1 mM Na2EDTA and 300 mM NaOH) (pH 13.0) for 20 minutes. Horizontal electrophoresis in fresh cold denaturation buffer was done at 300 mA and 25 V for 20 minutes. The slides were washed in neutralisation buffer (0.4 M Tris-HCl, pH 7.5) three times. Slides were stained with 50 µL of ethidium bromide solution (20 µgmL^{-1}, Sigma) (per slide), covered with cover slip and kept in container, in the dark conditions at 4°C.

The procedure was done under dimmed light, in order to avoid additional DNA damage caused by the exposure to the normal light. Each slide was examined using a 250x magnification fluorescence microscope (Zeiss, Germany) equipped with an excitation filter of 515-560 nm and a barrier filter of 590 nm. A total of 200 comets per sample and per interval were scored (100 from each of two replicate slides). Comets were randomly captured at a constant depth of the gel, avoiding the edges of the gel, occasional dead cells and superimposed comets. Using a black and white camera, the microscope image was transferred to a computer-based image analysis system (Comet Assay II, Perceptive Instruments Ltd., U.K.). To avoid the variability, one well-trained scorer scored all comets. Three parameters of DNA damage were analysed: tail length (TL, presented in micrometres), tail DNA (TI, %) and tail moment (TM).

2.2 DNA isolation

Genomic DNA was isolated from peripheral lymphocytes according to modified protocol by Daly et al. (1996) or according to protocol for genomic DNA lymphocyte isolation from QUIAGEN (mini KIT). DNA was purified with two times centrifugation at 4°C, 500 µL of 70 percent ethanol added every time. The pellet was dried overnight at room temperature and

diluted in 100 µL of TE buffer (10mM Tris–HCl, pH 7.4; 1 mM EDTA, pH 8.0). Purity and concentration of DNA was specified by spectrophotometric method (NanoDrop ND- 1000 spectrophotometer, NanoDrop Technologies, Thermo Scientific, Wilmington, USA). Samples were diluted till concentration of 10 ng µL^{-1} and kept at -20 °C till amplification. Specific polymorphisms were determined: in BER- (base excision repair) APE1- (apurinic/apirimidinic endonuclease, Asp148Glu), hOGG1 (human 8-oxoguanine DNA glycosylase, Ser326Cys), XRCC1 (X-ray repair cross-complementing protein-group 1, Arg194Trp); in NER- (nucleotide excision repair) XPD (Xeroderma pigmentosum-group D, Lys751Gln; DSBR- (double-strand-break repair) XRCC3 (X-ray repair cross-complementing protein-group 3, Thr241Met), PARP1 (poly (ADP-ribose) polymerase 1, Val762Ala); in DRR- (direct reversal repair) MGMT (O6-methylguanine-DNA methyltransferase, Leu84Phe) Genotyping was performed by either Real Time PCR (polymerase chain reaction) with Taqman assay, or after electrophoresis and fluorescence visualisation, DNA samples were cut with restriction enzymes.

2.3 Polymerase chain reaction-RFLP

In a total volume of 10 ml, 10 ng of genomic DNA was amplified for each sample. Compounds for the reaction mixture are given in Table 2.

PCR reaction was completed in six steps: (I) incubation at 94 °C (2 min) for Taq DNA polymerase activation; (II) incubation at 94 °C (30 s) for denaturation of double stranded DNA; (III) incubation at specific temperature that depended on the specific gene (30 s), for hybridisation of primers (Table 3); (IV) incubation at 72 °C (30 s) for DNA synthesis. Steps No.2 to No. 4 were repeated for 34 times. After that there were steps: (V) incubation at 72 °C (5 min) and (VI) incubation at 10 °C.

Reaction mixture	Stock solution	Final concentration for PCR reaction
(DMSO)	(1x)	(for XRCC3-0.05x)
reH$_2$O	1x	0.64x (for XRCC3-0.59x)
10X BUFFER	10x	1.00 x
MgCl$_2$	50 mM	2.00 mM
dNTP	1.25 mM	0.11 mM
Primer F	20 µM	0.30 µM
Primer R	20 µM	0.30 µM
DNA polymerase (Platinum Taq, Invitrogen)	5 U µl^{-1}	0,03 U µl^{-1}

Table 2. Compounds for PCR-RFLP reaction (10 µl of reaction mixture (9 µl of Master Mix and 1 µl of DNA sample).

GENE	rs	PCR method	primers	°C	Restriction enzyme
XPD ex 23 or ERCC2	rs13181 [Aag > Cag; Lys751Gln]	RFLP (Angelini et al., 2005)	F CCC CTC TCC CTT TCC TCT GTT R GCT GCC TTC TCC TGC GAT TA	61	PstI (37 °C, overnight) CTGCA*G G*ACGTC HOwt-29,244bp; HE-29,63,181,244bp; HOSNP-29,63,181bp
XPD10	rs1799793 [Gac > Aag; Asp312Asn]	Real Time TaqMan assay C_3145050_10	none	none	none
XRCC1 ex10	rs861539 [aCg > aTg; Trp194Arg]	RFLP (Angelini et al., 2005) RT TaqMan assay C_622564_10	F GCC CCT CAG ATC ACA CCT AAC R CAT TGC CCA GCA CAG GAT AA	61	MspI (37 °C, overnight) C*CGG GGC*C WT-161, 53 bp; HE-241, 161, 53 bp; SNP-214 bp
XRCC3 ex7	rs861539 [aCg > aTg; Thr241Met]	RFLP (Angelini et al., 2005)	F GCT CGC CTG GTG GTC ATC R CTT CCG CAT CCT GGC TAA AAA	60	Hin1 II/NIa III (37 °C, overnight)
hOGG1, ex4	rs1052133 [tCc > tGc; Ser326cys]	RFLP (Godderis et al., 2006) RT TaqMan assay C_3095552_1	F CCCAACCCAGTGGATTCTCATTGC R GGTGCCCCATCTAGCCTTGCGGCCCTT	60	SatI/ Fnu4HI (37 °C, overnight) GC*NGC CGN*CG HOwt-214,20pb; HE-214,165,49,20pb; HOSNP-165,49,20pb
PARP1	-	RT TaqMan assay	Real time	none	none
APE1	rs1130409 [gaT > gaG; Asp148Glu]	RFLP (Hu et al., 2002) RT TaqMan assay C_8921503_10	F CTGTTTCATTTCTATAGGCTA R AGGAACTTGCGAAAGGCTTC	57	BfaI (37 °C, overnight) (Asp/Asp 164bp, Asp/Glu 164,144,20bp; Glu/Glu-144,20bp)
MGMT C/T	rs12917, Leu84Phe	RT TaqMan assay	Real time	none	none

Table 3. Gene, forward and reverse primers, method used for determination of polymorphism, hybridisation temperature, restriction enzymes and the DNA fragments.

After PCR reaction, DNA products were cut with specific restriction enzymes (Fermentas, Vilnius, Litva) that have cut DNA samples on specific places and have given different DNA fragments in order to recognize which samples were homozygote wild type, heterozygote and polymorphic homozygotes. Treatment with restriction enzymes was performed at 37 °C in a period of 3 hours or overnight (due to the specification of specific restriction enzyme used in the reaction). Enzymes, time intervals of cutting, the temperature for those enzymes and the resulting DNA fragments after the cutting are given in Table 3. After electrophoresis that lasted 30 minutes at 200 V, PCR products were analysed on 10%-polyacrilamid gel (Bio-Rad, Hercules, USA).

Genotype results were regularly confirmed by random repetition of the samples.

PCR products were also amplified with Real-Time PCR (Real-Time PCR ABI Prism 7300 thermocycler, Applied Biosystems, Foster City, USA) with TaqMan allelic discrimination assay (Applera, Foster City, USA). Allelic determination was done by their software. Compounds for the Real-Time PCR mixture is shown in Table 4. Forty cycluses were performed for division between VIC and FAM fluorescence stain. The intensity of those stains is selecting the samples into three categories (Angelini et al., 2005).

Ingredients	Volume/μl
TaqMan Master Mix	6.5
reH$_2$O	5.33
Specific primers for every gene	0.65
DNA sample	0.5

Table 4. Compounds for Real Time PCR reaction. Steps in Real Time PCR-a were: (I) 95 °C (10 min); (II) 92 °C (15 s); and (III) 60 °C (10 min).

3. Results

DNA repair kinetics after the exposure to gamma radiation of 2 and 4 Gy was measured on a group of 126 subjects, 70 medical workers and 56 controls (Figure 1).

The groups differed in average age, gender, smoking status and alcohol consumption. The mean values for both groups did not significantly differ, although inter-individual differences were notable. In control group higher level of DNA damage compared to exposed group was observed, but without statistical difference. The repair dynamic was the same in both groups.

After genotyping, heterozygotes and polymorphic homozygotes were grouped together to evaluate polymorphic allele appearance. Number of individuals carrying particular gene is given in Table 5. Frequency of genotyping did not differ from expected Hardy-Weinberg equilibrium.
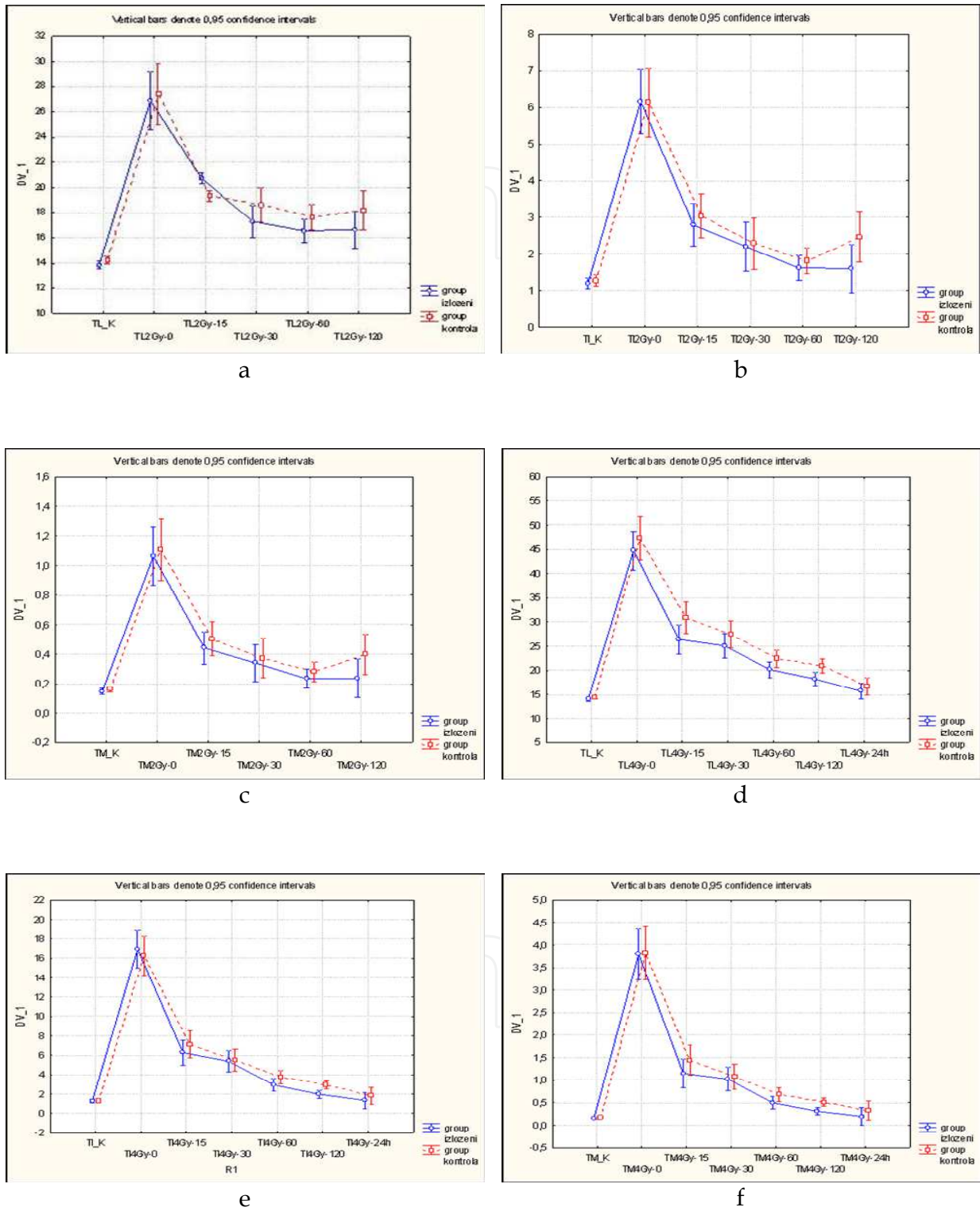
a

b

c

d

e

f

Fig. 1. Graphical results of DNA repair. Red colour - control group; blue - exposed group. a-TL after 2 Gy irradiation, b-TI after 2 Gy, c-TM after 2 Gy, d- TL after 4 Gy irradiation, e- TI after 4 Gy, f- TM after 4 Gy.

Gene	Group (WT/HE/SNP)	Control (WT/HE/SNP)	Exposed (WT/HE/SNP)
XPD 23	42/58/26	15/33/8	27/25/18
XPD 10	43/63/20	17/33/6	26/30/14
XRCC1	47/67/12	22/25/9	25/42/3
PARP1	83/37/6	33/18/5	50/19/1
HOGG1	78/43/5	42/14/0	36/29/5
APE1	43/57/26	19/27/10	24/30/16
MGMT C/T	87/34/5	39/16/1	48/18/4
XRCC3	45/61/20	23/26/7	22/35/13

Table 5. Number of individuals in three genotyping groups for all genes (WT-homozygote wild type, HE-heterozygote, SNP-polymorphic homozygote).

Variable	st	χ^2	p
XRCC1	1	0.1697	0.6804
hOGG1	1	7.3298	**0.0068**
APE2	1	0.0018	0.9665
XPD21	1	0.6372	0.4247
PARP2	1	2.1624	0.1414
MGMT C/T	1	0.0167	0.8971
XRCC3	1	1.6433	0.1999
smoking	1	1.7174	0.19
sex	1	13.2499	**0.0003**

Table 6. Results of χ2-test in the exposed and control group compared to genotype frequency, smoking and gender.

The differences between exposed and control group were analysed by χ2-test. Significant difference was found for hOGG1 gene and for gender (Table 6). Observed difference between smokers and non smokers was insignificant.

Multivariate regression analysis was used to estimate the influence of smoking, gender, age, years of exposure and genotypes on comet assay parameters immediately after and 120 minutes after exposure to 2 Gy and after 24 hours for the dose of 4 Gy.

Immediately after irradiation with the 2 Gy dose, exposed group had significantly lower amount of DNA damage than control group. Individuals with polymorphic variants of XRCC3 gene had higher TL than their homozygotes. Polymorphic variants of hOGG1 gene had higher TI than homozygotes. Polymorphic variants of hOGG1 gene had significantly higher TM than their homozygotes.

Tail length values significantly differed between the two groups both immediately after and also 120 minutes after irradiation with 2 Gy. Polymorphic variants of APE1 and XPD10 genes had higher TI and TM compared to homozygotes 120 min after irradiation with 2 Gy, and polymorphic variants of MGMT C/T and XRCC3 genes had significantly lower TI and TM than homozygotes (Table 7).

Variable	PE	SE	Parc R²	F	p
Log TL-2 Gy-0'					
Group	-0.10669	0.04189	0.0468	6.49	0.0124
XRCC3	0.09351	0.04390	0.0312	4.54	0.0357
MTR	0.07742	0.04335	0.0291	3.19	0.0772
Log TI-2 Gy-0'					
hOGG1	0.14426	0.07300	0.0509	3.91	0.0376
Log TM-2 Gy-0'					
TS	0.16398	0.10332	0.1157	2.52	0.0243
hOGG1	0.16083	0.09018	0.0776	3.18	0.0223
Log TL-2 Gy-120'					
Group	0.00069854	0.00019567	0.0914	12.74	0.0006
XPD 10	0.00037667	0.00020220	0.0274	3.47	0.0655
XRCC3	0.00031641	0.00020077	0.0220	2.48	0.1183
TI-2 Gy-120'					
Group	0.16440	0.04433	0.0004	13.75	0.1012
APE1	0.08068	0.04704	0.0896	2.94	0.0217
XPD10	0.09252	0.04627	0.0484	4.0	0.0239
MGMT C/T	-0.07375	0.04608	0.1128	2.56	0.0217
XRCC3	-0.09915	0.04763	0.0401	4.33	0.0262
TM-2 Gy-120'					
Group	0.48944	0.12713	0.0002	14.82	0.1075
APE1	0.23214	0.13490	0.0885	2.96	0.0217
XPD10	0.26994	0.13268	0.0447	4.14	0.0247
MGMT C/T	-0.20599	0.13216	0.1224	2.43	0.0204
XRRC3	-0.29847	0.13659	0.0313	4.77	0.0289

Table 7. Results of *stepwise* procedure in multivariate regression analysis for three comet assay parameters as a depended variable in the entire population immediately after radiation with 2 Gy dose and 120 minutes after irradiation (only the statistically significant results are shown in the Table).

Immediately after 4 Gy irradiation, multivariate regression analysis showed influence of smoking on TL. Polymorphic variants of SHMT1 genes had lower TL than their homozygotes. Gender had significant influence on TI. Polymorphic variants of SHMT1 and PARP1 genes had lower TI and TM when compared to their homozygotes.

Polymorphic variants of APE1 gene showed positive correlation with TL 24 hours after radiation with 4 Gy. Polymorphic variants of MGMT C/T gene had lower TL than homozygotes.

With the increase of age, significant increase in TI was measured 24 hours after exposure to 4 Gy. Polymorphic variants of PARP1 gene had lower values of TI than homozygotes. Age has shown positive correlation with TM. Lower values for TM were observed among polymorphic variants of PARP1 gene when compared to homozygotes (Table 8).

Variable	PE	SE	Parc R²	F	p
TL-4 Gy-0'					
Smoking	4.10520	2.19325	3.50	0.0642	0.0267
SHMT1	-3.74806	2.11391	3.14	0.0793	0.0297
TI-4 Gy-0'					
Gender	1.37561	0.94228	2.13	0.1475	0.0199
SHMT1	-1.65022	0.95455	2.99	0.0870	0.0294
PARP1	-1.63931	1.02226	2.57	0.1120	0.0257
TM-4 Gy-0'					
SHMT1	-0.14041	0.07716	3.31	0.0718	0.0304
PARP1	-0.13713	0.08267	2.75	0.1003	0.0260
TL-4 Gy-24h					
Gender	0.00036220	0.00017016	4.53	0.0361	0.0468
MTR	0.00047723	0.00017978	7.05	0.0094	0.0499
APE1	0.00036597	0.00018268	4.01	0.0483	0.0329
MGMT C/T	-0.00034719	0.00017942	3.74	0.0562	0.0359
TI-4 Gy-24h					
Gender	0.11087	0.04594	5.82	0.0180	0.0390
Age	0.00387	0.00203	3.63	0.0601	0.0376
TS	-0.07933	0.05406	2.15	0.1461	0.0741
MTHFR4	0.09859	0.04499	4.80	0.0313	0.0372
PARP1	-0.10466	0.04861	4.64	0.0342	0.0267
TM-4 Gy-24h					
Gender	2.03821	0.60467	11.36	0.0011	0.0869
Age	0.05384	0.02783	3.74	0.0565	0.0349
MTHFR4	1.59011	0.62442	6.48	0.0127	0.0635
MTR	0.97502	0.62696	2.42	0.1238	0.0224
PARP1	-1.21130	0.67510	3.22	0.0765	0.0313

Table 8. Results of *stepwise* procedure in multivariate regression analysis for three comet assay parameters as a depended variable in the entire population immediately after radiation with 4 Gy dose and 24 hours after irradiation (only the statistically significant results are shown in the Table).

3.1 Discussion

Development of new methods in genotoxicology, especially on molecular level, has greatly improved the knowledge and understanding of processes that follow after the organism was exposed to ionising radiation. In the same time, better radiation protection, that includes more sophisticated handling of radiation sources, better education of personnel who are operating on those sources, precise dosimetry and the use of protection equipment have reduced health risk in specific population occupationally exposed to ionising radiation. Recent investigations in the field of radiation protection have focused on individual differences in radiosensitivity. It has been shown that DNA repair capability is regulated with different mechanisms that include great number of genes involved directly or indirectly in the repair process.

DNA damage kinetics of primary damage can give us first information about the level of the damage. Estimation of damage after exposure to low doses of ionising radiation is possible in a very short time period (Olive et al., 1990, 1995; Tice et al., 1990). Most of the DNA breaks can be repaired during 30 minutes from the exposure to ionising radiation (Frankenberg-Schwager, 1989), and two hours from the exposure, almost all the damage is repaired (Plappert et al., 1997). Since the repair is so quick, estimation of DNA damage represents a major problem in studies of occupationally exposed professionals and demands developing of sensitive methods for detecting the level of DNA damage after exposure to low doses of ionising radiation.

In this study the influence of gene polymorphisms on DNA repair after exposure to 2 and 4 Gy of gamma radiation was investigated. The analysis of comet assay parameters did not give consistent results. Immediately after the irradiation with the dose of 2 Gy, values for tail intensity, which is recently considered the most reliable parameter for DNA damage estimation (Collins, 2004), were higher than the values of other authors for the same dose (Cornetta et al., 2006). The exposed and control group also had significantly higher TL and TM for all observed time intervals when compared to the control values before irradiation in both groups. On the other hand, TI significantly decreased after 30 minutes from the exposure. After 60 minutes from the exposure, the values did not significantly differ from the control values before irradiation in both control and exposed group. Those results were different that the ones found by Cornetta et al. (2006) during DNA repair assessment after the exposure to the dose of 2 Gy. They have shown that even after 60 minutes from the exposure, TI were still significantly higher than before radiation. The differences in significance in TL and TI in our research showed different sensitivity of those two parameters. Tail length values implicate the existence of small DNA fragments that have created comet tail shape during electrophoresis and showed the length of travelling of small fragments from the nucleus. On the other hand, TI show the amount of damaged DNA in comet tail. Damaged DNA can be seen as small DNA fragments or relaxed DNA loops from the comet head created during electrophoresis. Tail moment shows the ratio of DNA in comet tail. Our results have shown that most of the DNA damage has been repaired during 30 minutes from the exposure to 2 Gy. The amount of the unrepaired damage did not significantly differ from the amount of the damage in samples that were not irradiated, but were also investigated in DNA repair process. The results showed that TI was better marker of DNA damage than TL. Those findings are in agreement with results of Kumaravel & Jha (2006), who have also estimated the reliability of comet assay parameter after the exposure of peripheral blood samples to gamma irradiation with the doses of 0, 1, 2, 4 and 8 Gy. Besides the fact that TI and TM showed greater reliability for DNA damage estimation, they have also showed strong correlation with the received dose of irradiation.

After 4 Gy irradiation, the values for all three parameters in both control and exposed groups were higher than after irradiation with 2 Gy. Tail length values in irradiated samples were significantly higher than non-irradiated samples for the time periods of 0, 15, 30, 60 and 120 minutes, but not after 24 hours for both exposed and control group.

Values for TI and TM in irradiated samples were significantly different from the values of non-irradiated samples for time period of 0, 15, 30 and 60 minutes, suggesting that most of DNA damage has been repaired during that period, although the values measured 120 minutes and 24 hours after the exposure did not reach the values before the exposure in

exposed and control group. The results indicate the existence of small amount of DNA damage that is still present even after 24 hours from the exposure, independently of the dose level. The results could be explained by the difference in the repair of single stranded and double stranded DNA breaks. Single stranded DNA breaks are usually formed after the exposure to gamma or X-ray (90 %), while only small part of damage is created by double stranded breaks (10 %) (Cornetta et al., 2006). The repair of double stranded breaks after the exposure to ionising radiation usually lasts 12 or 16 hours (Vodicka et al., 2004), that is considerably longer than the repair of single stranded breaks. The amount of unrepaired damage in this experiment did not statistically differ from the control values before irradiation for both control and the exposed group. The difference between exposed and nonirradiated samples was higher for 2 Gy dose. The existence of small amount of unrepaired damage shows the importance of assessment of exposure to ionising radiation, especially to low doses and suggesting possible accumulation of primary, unrepaired damage that can lead to permanent damage and genome instability.

When the unrepaired amount of damage was compared, the results are implicating the existence of adaptive response in professionally exposed individuals to low doses of ionising radiation. Adaptive response in human lymphocytes has been described by other authors (Olivieri & Bosi, 1990; Sankaranarayanan et al., 1989; Shadley &Wolff, 1987; Wiencke et al., 1986; Wolff et al., 1990; Wolff, 1992, 1996). Aka et al. (2004) reported the existence of residual damage in tail intensity 60 and 120 minutes after the exposure to dose of 2 Gy. In their experiment, tail intensity values were also higher in the control group than the exposed one.

According to our results, despite the difference in the amount of damage, kinetics of repair after the exposure to both doses was similar to the one for the nonirradiated group. Unexpectedly, the difference between control and exposed group was significantly higher after 2 Gy than after 4 Gy exposure. These results were not in agreement with Wojcik et al. (1996) who claimed that kinetics of repair was faster in the exposed group. Aka et al. (2004) used comet assay for DNA repair assessment in human lymphocytes of male nuclear power plant workers and also for the control group (N=31). After the exposure, there was not significantly different repair kinetics between exposed and control group. Our results were comparable, indicating that healthy persons use the same repair mechanism, no matter if they are professionally exposed to ionising radiation or not. High standard deviation, also seen in the results of other author (DeMeo et al., 1991; Maluf et al., 2001; Maluf, 2004; Tice, 1995), implicated on the influence of interindividual differences. Since the comet assay measures primary DNA damage after the exposure, those results can be a marker of different gene activity. Besides the gene activity, the DNA repair can be influenced by the smoking status, gender, age and years of occupational exposure.

Smoking can also cause DNA damage when heavy and non heavy smokers were compared (Maluf et al., 2001; Maluf, 2004). Marcon et al. (2003) showed that heavy smokers can repair DNA damage after the exposure to 2 Gy faster than non smokers or non- heavy smokers. Olivieri et al. (1984) and Shadley (1994) suggested the existence of adaptive response that protects lymphocytes from oxidative damage. Smokers also showed lower levels of oxidised pyrimidinic bases in lymphocytes when compared to non-smokers (Berasati et al., 2001). Touil et al. (2002) showed that chemicals from the smoke can create covalent bonds between DNA and proteins, that can result with lower migration of DNA during electrophoresis,

showing false results, that is lower damage. Cebulska et al. (2007) showed negative influence of smoking on the efficiency of DNA repair in lymphocytes. On the other hand, Rzeszowska-Wolny et al. (2005) did not find significant correlation between smoking and DNA damage and DNA repair after the irradiation of human lymphocytes with the dose of 2 Gy. Our results also did not show the influence of smoking on DNA repair of primary damage, except for the tail length after the exposure to 4 Gy dose. The results are similar to Aka et al. (2004) on nuclear power plant workers.

It is still not clear whether the basal damage differ due to gender. Dusinska et al. (2004a) did not find the difference in creation of strand breaks, nor in the amount of DNA damage sensitive to action of specific enzymes such as endonuclease III, or FPG enzyme or alkali labile sites, although male had lower DNA damage after the treatment with hydrogen peroxide (Dusinska et al., 2004a, b). Bajpayee et al. (2002) showed that healthy men have more basal damage in lymphocytes than women. Our results showed that difference was only seen after the 4 Gy dose, where women had higher values for DNA damage in all three parameters observed in comet assay immediately after and 24 hours after the irradiation. There was not similar number of male and female, and that could also influence the results. Trzeciak et al. (2008) showed that there are differences in the amount of damage and the repair rate after the exposure of whole blood sample to 6.3 Gy dose. But most of the experiments did not show the gender influence on DNA repair capacity (Muller et al., 2001, 2002; Rajaee-Behbahani et al., 2001; Marcon et al., 2003).

Age influence was not seen in this experiment. That is in agreement with other authors (Betti et al., 1994; Frenzili et al., 1997). Rzeszowska-Wolny et al. (2005) did not show the influence of sex on DNA damage level and DNA repair by the comet assay after the exposure to 2 Gy dose. Maluf et al. (2001) showed positive correlation of age and DNA repair in the exposed group. Singh et al. (1988) described positive correlation between the age and DNA damage in non-smokers.

Although former results showed the existence of radio adaptive response, our results did not confirm the relationship between primary DNA damage and adaptive response. Few authors have also shown lower DNA damage levels after the repeated exposure to ionising radiation (Sankaranarayanan et al., 1989; Wang et al., 1991; Ikushima, 1992; Domingues et al., 1993).

APE1 gene is involved in BER repair. Polymorphism in exon 5 is connected with hypersensitivity during the exposure to ionising radiation (Au et al., 2006). Results are in agreement with Au et al. (2006). There was positive correlation of polymorphic variants and DNA damage level 120 minutes after the exposure to 2 Gy.

Polymorphic variants of hOGG1 gene showed positive correlation with TI and TM measured immediately after the exposure to the dose of 2 Gy. The results are in agreement with Aka et al. (2004) who showed the connection between polymorphic variants of hOGG1 Ser326Cys with lower capability of repair of oxidative DNA damage in the exposed, but not in the control group measured 60 and 120 minutes after the exposure to 2 Gy. After 120 minutes from the exposure, 71 % of individuals with high level of DNA damage had polymorphic variant of hOGG1 gene. Cornetta et al. (2006) did not show the influence of hOGG1 polymorphism on DNA damage.

Goode et al. (2002)) showed the connection between polymorphic variants of hOGG1 with higher risk of lung, head and neck cancer. hOGG1 and XRCC1 are involved in the same

repair pathway, while hOGG1 cuts oxidised base, XRCC1 together with ligase closes the break. Aka et al. (2004) and Matullo (2003) have described their connection. On the other hand, Cornetta et al. (2006), did not show significant difference between XRCC1, hOGG1 and XPC polymorphisms and DNA damage level measured 30 minutes from the exposure to 2 Gy dose. Yamane et al. (2004) showed that homozygotes differ for 2 fold factor in cutting of 8-oxodeoxiguanosine when compared to heterozygote.

XRCC1 polymorphism did not show the influence on DNA damage and repair, but polymorphic variants had lower levels of DNA damage for all three parameters measured. The results are not in agreement to those of Cornetta et al. (2006). Tail intensity values after the exposure to 2 Gy dose, immediatelly after, 30 minutes and 60 minutes after, homozygotes and polymorphic variants showed significant differences.

The two most common SNP polymorphisms in XPD gene are in exons 10 and 23. The results of studies of those polymorphisms are inconsistent. Naccarati et al. (2006) observed fewer number of single strand breaks in polymorphic homozygotes compared to heterozygotes and wild type of homozygotes. Similar results state Vodicka et al. (2004), analyzing chromosome aberrations frequency. Polymorphic XPD23 gene variants did not influence primary DNA damage, while polymorphic XPD10 gene variants showed positive correlation in all three comet assay parameters 120 min after exposure to 2 Gy of gamma radiation.

Polymorphic variants of XRCC3 in the exposed group had highest values for DNA damage. There was also positive correlation of polymorphic variants with TL immediately after the exposure to 2 Gy and negative correlation with TI and TL 120 minutes from the exposure to 2 Gy dose. Aka et al. (2004) did not find the correlation with primary damage, probably because this gene is involved in double strand breaks repair. PARP1 is important for integrity of chromosome ends and is involved in maintaining the integrity of BER and NHEJ repair. Our results showed negative correlation between polymorphic variants and TI values measured immediately after the exposure to 4 Gy and with TM values measured 24 hours after the exposure to 4 Gy.

MGMT C/T variants have higher risk of glioma (Liu et al., 2009), but there are no investigations that are connecting it with ionising radiation. In our study polymorphic variants have shown negative correlation with DNA damage level measured 120 minutes after the exposure to 2 Gy.

4. Conclusion

The results indicate that in addition to individual gene polymorphisms, the influence of combinations of polymorphic genes to DNA damage and repair should be tested.

5. Acknowledgment

6. References

Aka, P.; Mateuca, R.; Buchet, J.P.; Thierens, H. & Kirsch-Volders, M. (2004) Are genetic polymorphisms in OGG1, XRCC1 and XRCC3 genes predictive for the DNA strand

break repair phenotype and genotoxicity in workers xx)posed to low dose ionising radiations? *Mutat Res* 556: 169-181.

Andreassen, C.N. (2005) Can risk of radiotherapy-induced normal tissue complications be predicted from genetic profiles? *Acta Oncologica* 44: 801-815.

Andreassi, M.G.; Foffa, I.; Manfredi, S.; Botto, N.; Cioppa, A. & Picano, E. (2009) Genetic polymorphisms in XRCC1, OGG1, APE1 and XRCC3 DNA repair genes, ionizing radiation exposure and chromosomal DNA damage in interventional cardiologists. *Mutat Res* 666(1-2): 57-63.

Angelini, S.; Kumar, R.; Carbone, F.; Maffei, F.; Forti, G.C.; Violante, F.S.; Lodi, V.; Curti, S.; Hemminki, K. & Hrelia, P. (2005) Micronuclei in humans induced by exposure to low level of ionizing radiation: influence of polymorphims in DNA repair genes. *Mutat Res* 570: 105-117.

Ashby, J.; Tinwell, H.; Lefevre, P.A. & Browne, M.A. (1995) The single cell gel electrophoresis assay for induced DNA damage (comet assay): measurement of tail length and moment. *Mutagenesis* 10(2): 85-90.

Au, W.W. (1991) Monitoring human populations for effects of radiation and chemical exposures using cytogenetic techniques. *Occup Med* 4:597-611.

Au WW (2006) Heritable susceptibility factors for te development of cancer. *J Radiat Res* 47B: 13-17

Bailey, S. & Goodwin, E. (2004) DNA and telomeres: beginnings and endings. *Cytogenet Genome Res* 104: 109-115.

Bailey, S.; Cornforth, M.; Ullrich, R. & Goodwin, E. (2004) Dysfunctional mammalian telomeres join with DNA double-strand breaks. *DNA Repair* 3: 349-357.

Bajpayee, M.; Dhawan, A.; Parmar, D.; Pandey, A.K.; Mathur, N. & Seth, P.K. (2002) Gender-related differences in basal DNA damage inlymphocytes of a healthy Indian population using the alkaline Comet assay. *Mutat Res* 520: 83-91.

Balasem, A,N. & Ali, A.S.K. (1991) Establishment of dose–response relationships between doses of Cs137 gamma-rays and frequencies of micronuclei in human peripheral blood lymphocytes. *Mutat Res* 259:133–138.

Barquinero, J.F.; Barrios, L.; Caballin, M.R.; Miro, R.; Ribas, M.; Subias, A. & Egozcue, J. (1993) Cytogenetic analysis of lymphocytes from hospital workers occupationally exposed to low levels of ionizing radiation. *Mutat Res* 286: 275–279.

Barquinero, J.F.; Barrios, L.; Caballin, M.R.; Miro, R.; Ribas, M.; Subias, A. & Egozcue, J. (1995) Occupational exposure to radiation induces an adaptive response in human lymphocytes. *Int J Radiat Biol* 67 (2): 187-191.

Barquinero, J.F.; Barrios, L.; Caballin, M.R.; Miro, R.; Ribas, M.; Subias, A. & Egozcue, J. (1996) Decreased sensitivity to the cytogenetic effects of bleomycin in individuals occupationally exposed to ionizing radiation. *Mutat Res* 354:81-86.

Berasati Nia, A.; Van Schooten: F.J.; Schilderman, P.A.E.L.; De Kok, T.M.C.M.; Haenen, G.R.; Van Herwijnen, M.H.M.; Van Agen, E.; Pachen, D. & Kleinjans, J.C.S. (2001) A multi-biomarker approach to study the effects of smoking on oxidative DNA damage and repair and antioxidative defence mechanisms. *Carcinogenesis* 21: 395–401.

Berrington, A.; Darby, S.C.; Weiss, H.A. & Doll, R. (2001) 100 years of observation on British radiologists: mortality from cancer and other causes 1897–1997. *Br J Radiol* 34 74: 507–519.

Berwick, M. & Vineis, P. (2000) Markers of DNA repair and susceptibility to cancer in humans: An epidemiological review. *J Natl Cancer Inst* 91: 874-897.

Berwick, M. (2000) Gene-environment interaction in melanoma. *Forum Geneva* 10: 191– 200.

Betti, C.; Davini, T.; Gianessi, L.; Loprieno, N. & Barale, R. (1994) Microgel electrophoresis assay (comet test) and SCE analysis in human lymphocytes from 100 normal subjects. *Mutat Res* 307: 323-333.

Boffetta, P.; Caporaso, N.; Cuzick, J.; Lang, M.; Vineis, P.; Malats, N. & D'Errico, A. (1999) Metabolic polymorphisms and susceptibility to cancer. IARC Publications, 44 Lyon, France.

Bolus, N.E. (2001) Basic review of radiation biology and terminology. *J Nucl Med Technol* 46 29(2): 67-73. Review.

Bonassi, S.; Ugolini, D.; Kirsch-Volders, M.; Stromberg, U.; Vermeulen, R. & Tucker, J.D. (2005) Human population studies with cytogenetic biomarkers: review of the literature and future prospectives. *Environ Mol Mutagen* 45: 258–270.

Boothman, D.A.; Bouvard, I. & Hughes, E.N. (1989) Identification and characterisation of X-ray-induced proteins in human cells. *Cancer Res* 49 (11): 2871-2878.

Bosi, A. & Olivieri, G. (1989) Variability of the adaptive response to ionizing radiations in humans. *Mutat Res* 211(1): 13-17.

Boutcher, S. & Haas, T. (1985) External radiation doses to nuclear medicine technologists from procedures using 99mTc radiopharmaceuticals. *Can J Radiogr Radiother Nucl Med* 16: 161–165.

Cardisi, E.; Vrijheid, M.; Blettner, M.; Gilbert, E.; Hakama, M.; Hill, C.; Howe, G.; Kaldor, D.; Muirhead, C.R.; Schubauer-Berigan, M.; Yoshimura, T.; Bermann, F.; Cowper, G.; Fix, J.; Hacker, C.; Heinmiller, B.; Marshall, M.; Thierry-Chef, I.; Utterback, D.; Ahn, ,Y.O.; Amoros, E.; Ashmore, P.; Auvinen, A.; Bae, J.M.; Bernar Solano, J.; Biau, A.; Combalot, E.; Deboodt, P.; Diez Sacristan, A.; Eklof, M.; Engels, H.; Engholm, G.; Gulis, G.; Habib, R.; Holan, K;, Hyvonen, H.; Kerekes, A.; Kurtinaitis, J.; Malker, H.; Martuzzi, M.; Mastauskas, A.; Monnet, A.; Moser, M.; Pearce, M.S.; Richardson, D.B.; Rodriguez-Artalejo, F.; Rogel, A.; Tardy, H.; Telle-Lamberton, M.; Turai, I.; Usel, M. & Veress, K. (2005) Risk of cancer after low doses of ionising radiation: retrospective cohort study in 15 countries. *Br Med J* 331: 77–80.

Cardoso, R. S.; Takahashi-Hyodo, S.; Peitl Jr, P.; Ghilardi-Neto, T. & Sakamoto-Hojo, E.T. (2001) Evaluation of chromosomal aberrations, micronuclei, and sister chromatid exchanges in hospital workers chronically exposed to ionizing radiation. *Teratog Carcinog Mutagen* 21: 431–439.

Carrano, A.V. & Natarajan, A.T. (1988) Considerations for population monitoring using cytogenetic techniques. ICPEMC publication 14. *Mutat Res* 204: 379–406.

Cebulska-Wasilewska, A.; Pawłyk, I.; Panek, A.; Wiechec,´ A.; Kalina, I.; Popov, T.; Georgieva, T. & Farmer, P.B. (2007) Exposure to environmental polycyclic aromatic hydrocarbons: influences on cellularsusceptibility to DNA damage (sampling Kosice and Sofia). *Mutat Res* 620: 145–154.

Chen, S.; Tang, D.; Xue, K.; Xu, L.; Ma, G.; Hsu, Y. & Cho, S.S. (2002) DNA repair gene XRCC1 and XPD polymorphisms and risk of lung cancer in a Chinese population. *Carcinogenesis* 23: 1321-1325.

Coates, P.J.; Lorimore, S.A. & Wright, E.G. (2004) Damaging and protective cell signalling in the untargeted effects of ionizing radiation. *Mutat Res* 568: 5-20.

Collins, A. & Harrington, V. (2002) Repair of oxidative DNA damage: assessing its contribution to cancer prevention. *Mutagenesis* 17: 489-493.

Collins, A.R. (2004) The Comet Assay for DNA damage and repair: Principles, applications and limitations. *Mol Biotechnol* 26: 249-261.

Cornetta, T.; Festa, F.; Testa, A. & Cozzi, R. (2006) DNA damage repair and genetic polimorphisms: assessment of indivisual sensitivity and repair capacity. *Int J Radiation Oncology Biol Phys* 66 (2): 537-545.

Daly, A.K.; Steen, V.M.; Fairbrother, K.S. & Idle, J.R. (1996) CYP2D6 multiallelism. *Meth Enzymol* 272: 199–210.

DeMeo, M.; Laget, M.; Castegnaro, M. & Dumenil, G. (1991) Genotoxic activity of potassium permanganate in acidic solutions. *Mutat Res* 260: 295-306.

Divine, K.K.; Gilliland, F.D.; Crowell, R.E.; Stidley, C.A.; Bocklage, T.J.; Cook, D.L. & Belinsky, S.A. (2001) The XRCC1 399 glutamine allele is a risk factor for adenocarcinoma of the lung. *Mutat Res* 461: 273-278.

Domingues, I; Panneerselvam, N.; Escalza, P.; Natarajan, A.T. & Cortes, F. (1993) Adaptive response to radiation damage in human lymphocytes conditioned with hydrogen peroxide as measured by the cytokinesis-block micronucleus technique. *Mutat Res* 301: 135–141.

Dusinska, M.; Collins, A.; Kazimirova, A.; Barancokova, M.; Harrington, V.; Volkovova, K.; Staruchova, M.; Horska, A.; Wsolova, L.; Kocan, A.; Petrik, J.; Machata, M.; Ratcliffe, B. & Kyrtopoulos, S. (2004a) Genotoxic effects of asbestos in humans. *Mutat Res* 553: 91–102.

Dusinska, M.; Kazimirova, A.; Barancokova, M.; Horska, A.; Burghardtova, K.; Volkovova, K.; Staruchova, M.; Wsolova, L. & Collins, A.R. (2004b) Does occupational exposure to mineral fibres cause DNA or chromosome damage? *Mutat Res* 553: 103–110.

Erexon, G.L.; Kligerman, A.D.; Bryant, M.F.; Sontag, M.R. & Halperin, E.C. (1991) Induction of micronuclei by X-radiation in human, mouse and rat peripheral blood lymphocytes. *Mutat Res* 253: 193–198.

Fachini, A.L.; Mello, S.S.; Sandrin-Garcia, P.; Junta, C.M.; Ghilardi-Netto, T.; Donadi, E.A.; Da Silva, G.A.; Elza, P. & Sakamoto-Hojo, T. (2009) Gene expression profiles in radiation workers occupationally exposed to ionizing radiation. *J Radiat Res* 50: 61–71.

Fairbairn, D.W.; Olive, P.L.; O´Neill, K.L. (1995) The comet assay: a comprehensive review. *Mutat Res* 339: 37-59.

Fenech, M.; Denham, J.; Francis, W. & Morley, A.A. (1990) Micronuclei in cytokinesis blocked lymphocytes of cancer patients following fractionated partial-body radiotherapy. *Int J Radiat Biol* 57: 373–383.

Frankenberg-Schwager, M. (1989) Review of repair kinetics for DNA damage induced in eukaryotic ce,lls in vitro by ionizing radiation. *Radiother Oncol* 14: 307-320.

Frenzili, G.; Betti, C.; Davini, T.; Desideri, M.; Fornai, E.; Gianessi, L.; Maggiorelli, F.; Paoletti, P. & Barale, R. (1997) Evaluation of DNA damage in leukocytes of ex smokers by single cell gel electrophoresis. *Mutat Res* 375: 117-123.

Goode E.L-; Ulrich C.M. & Potter J.D. (2002) Polymorphisms in DNA repair genes and associations with cancer risk. *Cancer Epidemiol Biomarkers Prev* 11: 1513–1530.

Gourabi, H. & Mozdarani, H. (1998) A cytokinesis-blocked micronucleus study of the radioadaptive response of lymphocytes of individuals occupationally exposed to chronic doses of radiation. *Mutagenesis* 13(5): 475-480.

Hall, E.J. & Giaccia, A.J. (2006) Radiobiology for the Radiologist. 6th edn. Lippincott Williams & Wilkins, Philadelphia

He, J.L.; Chen, W.L.; Jin, L.F. & Jin, H.Y. (2000) Comparative evaluation of the in vitro micronucleus test and comet assay for the detection of genotoxic effects of X-ray radiation. *Mutat Res* 469:223–231.

Hellman, B.; Vaghef, H. & Bostrom, B. (1995) The concepts of tail moment and tail inertia in the single cell gel electrophoresis assay. *Mutat Res* 336: 123-131.

Hellman, B.; Vaghef, H.; Friis, L. & Edling, C. (1997) Alkaline single cell gel electrophoresis of DNA fragments in biomonitoring for genotoxicity: an introductiory study on healthy human volunteers. *International Archives of Occupational and Environmental Health* 69: 185-192.

Hinds, D.A.; Stuve, L.L.; Nilsen, G.B.; Halperin, E.; Eskin, E.; Ballinger, D.G.; Frazer, K.A. & Cox, D.R. (2005) Whole genome patterns of common DNA variation in three human populations. *Science* 307: 1072-1079.

Hoffmann H. & Speit G. (2005) Assessment of DNA damage in peripheral blood of heavy smokers with the comet assay and the micronucleus test. *Mutat Res* 581:105-14.

Hou, S.M.; Falt, S.; Angelini, S.; Yang, K.; Nyberg, F.; Lambert, B. & Hemminki, K. (2002) The XPD variant alleles are associated with increased aromatic DNA adduct level and lung cancer risk. *Carcinogenesis* 23: 599-603.

Hu, J.J.; Smith, T.R.; Miller, M.S.; Mohrenweiser, H.W.; Golden, A. & Case, L.D. (2001) Amino acid substitution variants of APE1 and XRCC1 genes associated with ionizing radiation sensitivity. *Carcinogenesis* 22: 917–922.

Hu, J.J.; Mohrenweiser, H.W.; Bell, D.A.; Leadon, S.A. & Miller, M.S. (2002a) Symposium overview: genetic polymorphisms in DNA repair and cancer risk. *Toxicol Appl Pharmacol* 185: 64–73.

Hu, J.J.; Smith, T.R.; Miller, M.S.; Lohman, K. & Case, L.D. (2002b) Genetic regulation of ionizing radiation sensitivity and breast cancer risk. *Environ Mol Mutagen* 39: 208-215.

Hu, Z.; Ma, H.; Chen, F.; Wei, Q. & Shen, H. (2005) XRCC1 polymorphisms and cancer risk: a meta-analysis of 38 case-control studies. *Cancer Epidemiol Biomarkers Prevent* 14:1810–1818.

Hung, R.J.; Hall, J.; Brennan, P. & Boffetta, P. (2005) Genetic polymorphisms in the base excision repair pathway and cancer risk: a HuGE review. *Am J Epidemiol* 162(10): 21 925-942.

Ikushima, T. (1989) Radio-Adaptive Response: Characterization of a cytogenetic repair induced by low-level ionizing radiation in cultured Chinese hamster cells. *Mutat Res* 227 (4): 241-246.

Ikushima, T. (1992) Radio-adaptive response: involvement of induction of specific gene expression by low doses of ionizing radiation. In: Sugahara T, Sagan L, Aoyama T(ed.) Low Dose Irradiation and Biological Defence Mechanisms. Elsevier Science Publishers BV, Amsterdam, 255–263.

Ikushima, T.; Aritomi, H. & Morisita, J. (1996) Radioadaptive Response: Efficient repair of radiation-induced DNA damage in adapted cells. *Mutat Res* 358(2):193-198.

Jeggo, P. & Lavin, M.F. (2009) Cellular radiosensitivity: how much better do we understand it? *Int J Radiat Biol* 85:1061-1081.

Jha, A.N. & Sharma, T. (1991) Enhanced frequency of chromosome aberrations in workers occupationally exposed to diagnostic X-rays. *Mutat Res* 260: 343–348.

Kassie, F.; Parzefall, W. & Knasmuller, S. (2000) Single cell gel electrophoresis assay: new technique for human biomonitoring studies. *Mutat Res* 463: 13–31.

Kotsopoulos, J.; Chen, Z.; Vallis, K.A.; Poll, A.; Ainsworth, P. & Narod, S.A. (2007) DNA repair capacity as a possible biomarker of breast cancer risk in female BRCA1 mutation carriers. *Br J Cancer* 96:118–125.

Kruszewski, M.; Wojewodzka, M.; Iwanenko, T.; Collins, A.R. & Szumiel, I. (1998) Application of the comet assay for monitoring DNA damage in workers exposed to chronic low-dose irradiation: II. Base damage. *Mutat Res* 416: 37–57.

Kubelka, D.; Sviličić, N.; Kralik, I.; Belamarić, N.; Faj, D.; Novosel, N.; Vukić, V. & Tečić Z. (2011) .Analiza učinkovitosti i usklanenosti s EU propisima zakonodavstva kojim se regulira radiološka i nuklearna sigurnost [Analysis of efficacy and conformity with the EU acquis communautaire of the radiation protection and nuclear safety legislative; *in Croatian*]. In: Krajcar Bronić I, Kopjar N, Milić M, 1 Branica G, editors. Proceedings of the 8th Symposium of the Croatian Radiation Protection Association; 13-15 Apr 2011; Krk, Croatia. Zagreb: Denona; p. 31-37.

Kumar, R.; Hoglund, L.; Zhao, Ch.; Forsti, A.; Snellman, E. & Hemminki, K. (2003) Single nucleotide polymorphisms in the XPG gene: determination of role in DNA repair and breast cancer risk. *Int J Cancer* 103: 671-675.

Kumaravel, T.S. & Jha, A.N. (2006) Reliable Comet assay measurements for detecting DNA damage induced by ionising radiation and chemicals. *Mutat Res* 605: 7-16.

Little, J.B. (2000) Radiation carcinogenesis. *Carcinogenesis* 21: 397–404.

Liu, Y.; Scheurer, M.E.; El-Zein, R.; Cao, Y.; Do, K.A.; Gilbert, M.; Aldape, K.D.; Wei, Q.; Etzel, C. & Bondy, M.L. (2009) Association and interactions between DNA repair gene polymorphisms and adult glioma. *Cancer Epidemiol Biomarkers Prev* 18(1): 204-214

Lunn, R.M.; Helzlsouer, K.J.; Parshad, R.; Umbach, M.D.; Harris, E.L.; Sanford, K.K. & Bell D.A. (2000) XPD polymorphisms: effects on DNA repair proficiency. *Carcinogenesis* 21: 551–555.

Maitre, A.; Colonna, M.; Gressin, C.; Menegoz, F. & de Gaudemaris, R. (2003) Increased incidence of haematological cancer among physicians in a University Hospital. *Int Arch Occup Environ Health* 76: 24–28.

Maluf, S. & Erdtmann, B. (2001a) Genomic instability in Down syndrome and Fanconi anemia assessed by micronucleus analysis and single-cell gel electrophoresis. *Cancer Genet Cytogenet* 124: 71–75.

Maluf, S.W.; Passos, D.F.; Bacelar, A.; Speit, G. & Erdtmann, B. (2001b) Assessment of DNA damage in lymphocytes of workers exposed to X-radiation using the micronucleus test and the comet assay. *Environ Mol Mutagen* 38: 311–315

Maluf, S.W. (2004) Monitoring DNA damage following radiation exposure using cytokinesis-block micronucleus method and alkaline single-cell gel electrophoresis. *Clinica Chimica Acta* 347: 15-24.

Marcon, F.; Andreoli, C.; Rossi, S.; Verdina, A.; Galati, R. & Crebelli, R. (2003) Assessment of individual sensitivity to ionizing radiation and DNA repair efficiency in a healthy population. *Mutation Research* 541: 1–8.

Matullo, G.; Peluso, M.; Polidoro, S.; Guarrera, S.; Munnia, A.; Krogh, V.; Masala, G.; Berrino, F.; Panico, S.; Tumino, R.; Vineis, P. & Palli, D. (2003) Combination of DNA repair gene single nucleotidepolymorphisms and increased levels of DNA adducts in a population-based study. *Cancer Epidemiol. Biomarkers Prev* 12: 674–677

McKelvey-Martin, V.J.; Green, M.H.L.; Schmezer, P.; Pool-Zobel, B.L.; De Me´o, M.P. & Collins, A. (1993) The single cell gel electrophoresis assay (comet assay): a European review. *Mutat Res* 288: 47–63.

McKelvey-Martin, V.J.; Ho, E.T.S.; McKeown, S.R.; Johnston, S.R.; McCarthy, P.J.; Rajab, N.F. & Downes, C.S. (1998) Emerging applications of the single cell gel electrophoresis (Comet) assay. I. Management of invasive transitionalcell human bladder carcinoma. II. Fluorescent in situ hybridization Comets for the identification of damaged and repaired DNA sequences in individual cells. *Mutagenesis* 13: 1–8.

Milić, M.; Rozgaj, R.; Kašuba, V.; Kubelka, D.; Angelini, S. & Hrelia, P. (2010) DNA repair genes and radiation sensitivity. *Arh Hig Rada Toksikol* 61 Supplement: 107-121.

Milić, M. (2010) Važnost individualne osjetljivosti za procjenu rizika od oštećenja genoma pri kroničnoj profesionalnoj izloženosti niskim dozama ionizirajućeg zračenja [The significance of individual genome sensitivity in risk assessment of chronic professional exposure to low doses of ionizing radiation; in Croatian]. [PhD thesis]. Zagreb: Faculty of Science, University of Zagreb.

Mohrenweiser, H.W. & Jones, I.M. (1998) Variation in DNA repair is 1 a factor in cancer susceptibility: a paradigm for the promises and perils of individual and population risk estimation? *Mutat Res* 400: 15–24.

Mohrenweiser, H.W.; Wilson, D.M. & Jones, I.M. (2003) Challenges and complexities in estimating both the functional impact and the disease risk associated with the extensive genetic variation in human DNA repair genes. *Mutat Res* 526: 93–125.

Monsieurs, M.A.; Thierens, H.M.; Vral, A.M.; Van De Wiele, C.; De Ridder, L.I. & Dierckx, R.A. (2000) Adaptive response in patients treated with 131I. *J Nucl Med* 41 (1): 17-22.

Morgan, W.F. (2003) Non-targeted and delayed effects of exposure to ionizing radiation: i. radiation-induced genomic instability and bystander effects *in vitro. Radiat Res* 159: 567-580.

Mothersill, C.; Kadhim, M.A.; O' Reilly, S.; Paphworth, D.; Mardsen, S.J.; Seaymoure, C.B. & Wright, E.G. (2000) Dose- and time-response relationships for lethal mutations and chromosomal instability induced by ionizing radiation in an immortalized human keratinocyte cell line. *Int Radiat Biol* 76 (6): 799-806.

Mothersill, C.; Rea, D.; Wright, E.G.; Lorimore, S.A.; Murphy, D.; Seymour, C.B. & O'Malley, K. (2001) Individual variation in the production of a bystander signal following irradiation of primary cultures of normal human urothelium. *Carcinogenesis* 22 (9): 1465-1471.

Muller, W.U.; Bauch, T.; Stuben, G.; Sack, H. & Streffer, C. (2001) Radiation sensitivity of lymphocytes from healthy individuals and cancer patients as measured by the comet assay. *Radiat Environ Biophys* 40: 83–89.

Muller, W.U.; Bauch, T.; Streffer, C. & von Mallek, D. (2002) Does radiotherapy affect the outcome of the comet assay? *Br J Radiol* 75: 608–614.

Naccarati, A.; Soucek, P.; Stetina, R.; Haufroid, V.; Kumar, R.; Vodickova, L.; Trtkova, K.; Dusinska, M.; Hemminki, K. & Vodicka, P. (2006) Genetic polymorphisms and possible gene-gene interactions in metabolic andDNA repair genes: Effects on DNA damage. *Mutat Res* 593: 22–31

Norppa, H. (2004) Cytogenetic biomarkers and genetic polymorphisms. *Toxicol Lett* 149: 309–334.

Nowak, B. & Jankowski, J. (1991) Occupational exposure in operational radiology. *Pol J Occup Med Environ Health* 4: 169–174.

Okayasu, R.; Suetomi, K.; Yu, Y.; Silver, A.; Bedford, J.; Cox, R. & Ullrich, R. (2000) A deficiency in DNA repair and DNA-PKcs expression in the radiosensitive BALB/c mouse. *Cancer Res*. 60: 4342-4345.

Olive, P.L.; Banath, J.P. & Durand, R.E. (1990) Heterogeneity in radiation-induced DNA damage and repair in tumor and normal cells using the "comet" assay. *Radiation Research* 122: 86-94.

Olive, P.L. (1999) DNA damage and repair in individual cells: aplications of the comet assay in radiobiology. *International Journal of Radiation Biology* 75(4): 395-405.

Olivieri, G.; Bodycote, J. & Wolff, S. (1984) Adaptive response of human lymphocytes to low concentrations of radioactive thymidine. *Science* 223: 594–597.

Olivieri, G. & Bosi, A. (1990) Possible causes of variability of the adaptive response in human lymphocytes. In: Obe G, Natarajan A T(ed.) Chromosomal Aberrations, Basic and Applied Aspects. Springer-Verlag, Berlin, 130–140.

Padovani, L.; Appolloni, M.; Anzidei, P.; Tedeschi, B.; Caporossi, D.; Vernole, P. & Mauro, F. (1995) Do human lymphocytes exposed to the fallout of the Chernobyl accident exhibit an adaptive response? I. Challenge with Ionizing Radiation. *Mutat Res* 332 (1-2): 33-38.

Parl, F.F. (2005) Glutathione S-transferase genotypes and cancer 1 risk. *Cancer Lett* 221:123–129.

Plappert, U.; Raddatz, K.; Roth, S. & Fliedner, T.M. (1995) DNA damage detection in man after radiation exposure-the comet assay- its possible application for human monitoring. *Stem Cells* 13(1): 215-222.

Plappert, U.G.; Stocker, B.; Fender, H. & Fliedner, T.M. (1997) Changes in the repair capacity of blood cells as a biomarker for chronic low-dose exposure to ionizing radiation. *Environ Mol Mutagen* 30(2): 153-160.

Price, A. (1993) The repair of ionising radiation- induced damage to DNA. *Cancer Biology* 4: 61-71.

Rajaee-Behbahani, N.; Schmezer, P.; Risch, A.; Rittgen, W; Kayser, K.W.; Dienemann, H.; Schulz, V.; Drings, P., Thiel, S. & Bartsch, H. (2001) Altered DNA repair capacity and bleomycin sensitivity as risk markers for non-small cell lung cancer. *Int J Cancer* 95: 86–91.

Robson, T., Joiner, M.C.; Wilson, G.D.; McCullough, W.; Price, M.E.; Logan, I.; Jones, H.; McKeown, S.R. & Hirst, D.G. (1999) A novel human stress response-related gene with a potential role in induced radioresistance. *Radiat Res* 152 (5): 451-461.

Roos, W.P.; Binder, A. & Bohm, L. (2000) Determination of the initial DNA damage and residual DNA damage remaining after 12 hours of repair in eleven cell lines at low doses of irradiation. *Int J Radiat Biol* 76: 1493-1500.

Rzeszowska-Wolny, J.; Polanska, J.; Pietrowska, M.; Palyvoda, O.; Jaworska, J.; Butkiewicz, D. & Hancock, R. (2005) Influence of polymorphisms in DNA repair genes XPD, XRCC1 and MGMT on DNA damage induced by gamma radiation and its repair in lymphocytes *in vitro*. *Radiat Res* 164: 132–140.

Sankaranarayanan, K.; Duyn, A.; Loos, M.J. & Natarajan, A.T. (1989) Adaptive response of human lymphocytes to low-level radiation from radioisotopes or X-rays. *Mutat Res* 211: 7–12.

Sari-Minodier, I.; Orsiere, T.; Auquier, P.; Martin, F. & Botta, A. (2007) Cytogenetic monitoring by use of the micronucleus assay among hospital workers exposed to low doses of ionizing radiation. *Mutat Res* 629(2): 111-121.

Sasaki, M.S.; Ejima, Y.; Tachibana, A.; Yamada, T.; Ishizaki, K.; Shimizu, T. & Nomura, T. (2002) DNA damage response pathway in radioadaptive response. *Mutat Res* 33 504: 101-118.

Setlow, R.B. (1983) Variations in DNA repair among humans. In: Harris CC, Autrup HN (ed.) Human Carcinogenesis. New York: Academic Press: 231-254.

Seymour, C.B. & Mothersill, C. (2000) Relative contribution of bystander and targeted cell killing to the low-dose region of the radiation dose-response curve. *Radiat Res* 153: 508-511.

Shadley, J.D. & Wiencke, J.K. (1989) Induction of the adaptive response by x-rays is dependent on radiation intensity. *Int J Radiat Biol* 56: 107-118.

Shadley, J.D. (1994) Chromosomal adaptive response in human lymphocytes. *Radiat Res* 138: S9–S12.

Shadley, J.D. & Wolff, S. (1987) Very low doses of X-rays can cause human lymphocytes to become less susceptible to ionizing radiation. *Mutagenesis* 2: 95–96.

Shastry, B.S. (2009) SNPs: impact on gene function and phenotype. *Methods Mol Biol* 578: 3-22.

Singh, N.P.; McCoy, M.T.; Tice, R.R. & Schneider, L.L. (1988) A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp Cell Res* 175:184-191.

Singh, N.P. (2000) Microgels for estimation of DNA strand breaks, DNA protein crosslinks and apoptosis. *Mutat Res* 455: 111-127.

Stecca, C. & Gerber, G.B. (1998) adaptive response to dna-damaging agents: a review of potential mechanisms. *Biochem Pharmacol* 55 (7): 941-951.

Sturgis, E.M.; Castillo, E.J.; Li, L.; Zheng, R.; Eicher, S.A.; Clayman, G.L.; Strom, S.S.; Spitz, M.R. & Wei, Q. (1999) Polymorphisms of DNA repair gene XRCC1 in squamous cell carcinoma of the head and neck. *Carcinogenesis* 20: 2125-2129.

Szumiel, I. (1998) Monitoring and signaling of radiation-induced damage in mammalian cells. *Radiat Res* 150: 92-101.

Tedeschi, B.; Caporossi, D.; Vernole, P.; Padovani, L.; Appolloni, M.; Anzidei, P. & Mauro, F. (1995) Do Human lymphocytes exposed to the fallout of the Chernobyl accident exhibit an adaptive response? II. Challenge with bleomycin. *Mutat Res* 332 (1-2): 299-300.

Tedeschi, B.; Caporossi, D.; Vernole, P.; Padovani, L. & Mauro, F. (1996) Do human lymphocytes exposed to the fallout of the chernobyl accident exhibit an adaptive response? III. Challenge with bleomycin in lymphocytes from children hit by the initial acute dose of ionizing radiation. *Mutat Res* 354 (1): 77-80.

Tice, R.R.;Andrews, P.W.; Hirai, O. & Singh, N.P. (1990) The single cell gel (SCG) assay: an electrophoretic technique for the detection of DN damage in individual cells. In: Witmer, C.M., Snyder, R.R., Hollow, D.J., Kalf, G.F., Kocsis, J.J., Sipes, J.G. (ed.) Biological reactive intermediates, IV Molecular and cellular effects and their impact on human health. New York, Plenum Press, 157-164.

Tice, R.R. (1995) The single cell gel/comet assay: a microgel electrophoretic technique for the detection of DNA damage and repair in individual cells. In: Phillis DH, Venitt S (ed.) *Environmental Mutagenesis*. Bioscientific, Oxford, 315-339.

Touil, N.; Aka, P.V.; Buchet, J.P.; Thierens, H. & Kirsch-Volders, M. (2002) Assessment of genotoxic effects related to chronic low level exposureto ionizing radiation using biomarkers for DNA damage and repair. *Mutagenesis* 17: 223–232.

Trzeciak, A.R.; Barnes, J.; Ejiogu, N.; Foster, K.; Brant, L.J.; Zonderman, A.B. & Evans, M.K. (2008) Age, sex, and race influence single-strand break repair capacity in a human population. *Free Radic Biol Med* 45:1631-1641.

United Nations Scientific Committee on the Effects of Atomic Radiation, Sources and effects of ionizing radiation UNSCEAR 2000 report, vol. 1, http://www.unscear.org. UNSCEAR (United Nations Scientific Committee on theEffects of Atomic Radiation), 2000. Sources and effects of ionizing radiation. Report to the General Assembly.

Valverde, M.; Ostrosky-Wegman, P.; Rojas, E.; Fortoul, T.; Meneses, F.; Ramírez, M.; Díaz-Barriga, F. & Cebrian, M. The application of single cell gel electrophoresis or Comet assay to human monitoring studies. *Salud Publica Mex.* 1999;41 Suppl 2:S109-13.

Vodicka, P.; Kumar, R.; Stetina, R.; Sanyal, S.; Soucek, P.; Haufroid, V.; Dusinska, M.; Kuricova, M.; Zamecnikova, M., Musak, L.; Buchancova, J.; Norppa, H.; Hirvonen, A.; Vodickova, L.; Naccarati, A.; Matousu, Z. & Hemminki, K. (2004) Genetic polymorphismsin DNA repair genes and possible links with DNA repair rates,

chromosomal aberrations and single-strand breaks in DNA. *Carcinogenesis* 25: 757–763.

Wang, J.X.; Zhang, L.A.; Li, B.X.; Zhao, Y.C.; Wang, Z.Q.; Zhang, J.Y. & Aoyama, T. (2002) Cancer incidence and risk estimation among medical X-ray workers in China, 1950–1995, *Health Phys* 82: 455–466.

Wang, Z.Q.; Saigusa, S. & Sasaki, M.S. (1991) Adaptive response to chromosome damage in cultured human lymphocytes primed with low doses of X-rays. *Mutat Res* 246: 179–186.

Weiss, J.M.; Goode, E.L.; Ladiges, W.C. & Ulrich, C.M. (2005) Polymorphic variation in hOGG1 and risk of cancer: a review of the functional and epidemiologic literature. *Mol Carcinog* 42: 127–141.

Wiencke, J.K.; Afzal, V; Olivieri, G. & Wolff, S. (1986) Evidence that the (3h) thymidine induced adaptive response of human lymphocytes to subsequent doses of x-rays involves the induction of a chromosomal repair mechanism. *Mutagenesis* 1(5): 375-380.

Winsey, S.L.; Haldar, N.A.; Marsh, H.P.; Bunce, M.; Marshall, S.E.; Harris, A.L.; Wojnarowska, F. & Welsh, K.I. (2000) A variant within theDNA repair gene XRCC3 is associated with the development of melanoma skin cancer. *Cancer Res* 60: 5612-5616.

Wojcik, A.; Sauer, K.; Zolzer, F.; Bauch, T. & Muller, W.U. (1996) Analyses of DNA damage recovery processes in the adaptive response to ionising radiation in human lymphocytes. *Mutagenesis* 11: 291–297.

Wojewodzka, M.; Kruszewski, M.; Iwanenko, T.; Collins, A.R. & Szumiel, I. (1998) Application of the comet assay for monitoring DNA damage inworkers exposed to chronic low-dose irradiation: I. Strand breakage. *Mutat Res* 416:21–35.

Wolff, S.; Wiencke, J.K. & Afzal, V. (1989) Biological Bases of Risk Assessment. In: Baverstock KF and Stather JW (ed.) Low Dose Radiation. Taylor and Francis, London, 439-445.

Wolff, S.; Oliviery, G. & Afzal, V. (1990) Adaptation of human lymphocytes to radiation or chemical mutagens: differences in cytogenetic repair. In: Obe G., Natarajan, A.T. (ed.) Chromosomal Aberrations, Basic and Applied Aspects. Springer-Verlag, Berlin, 140–149.

Wolff, S. (1992) Low-dose exposures and the induction of adaptation. In: Sugahara, T., Sagan, L.A., Aoyama, T. (ed.) Low Dose Irradiation and Biological Defence Mechanisms. Elsevier Science Publishers BV, Amsterdam, 21–28.

Wolff, S. (1996) Aspects of the adaptive response to very low doses of radiation and other agents. *Mutat Res* 358: 135–142.

Yamane, A.; Kohno, T.; Ito, K.; Sunaga, N.; Aoki, K.; Yoshimura, K.; Murakami, H.; Nojima, Y. & Yokota, J. (2004) Differential ability of polymorphic OGG1 proteins to suppress mutagenesis induced by 8-hydroxyguanine in human cell in vivo. *Carcinogenesis* 25: 1689-1694

Yoshinaga, S.; Aoyama, T.; Yoshimoto, Y. & Sugahara, T. (1999) Cancer mortality among radiological technologists in Japan: updated analysis of follow-up data from 1969 to 1993. *J Epidemiol* 9: 61–72.

Youngblom, J.H.; Wiencke, J.K. & Wolff, S. (1989) Inhibition of the adaptive response of human lymphocytes to very low doses of ionizing radiation by the protein synthesis inhibitor cycloheximide. *Mutat Res* 227(4):257-261.
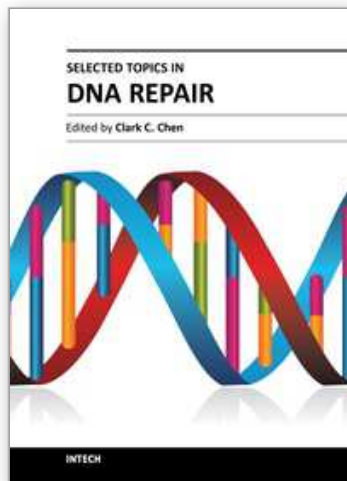
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