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Polymorphisms in DNA repair genes: link with biomarkers of the CBMN cytome assay in hospital workers chronically exposed to low doses of ionising radiation

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Individual sensitivity to ionising radiation (IR) is the result of interaction between exposure, DNA damage, and its repair, which is why polymorphisms in DNA repair genes could play an important role. We examined the association between DNA damage, expressed as micronuclei (MNi), nuclear buds (NBs), and nucleoplasmic bridges (NPBs) and single nucleotide polymorphisms in selected DNA repair genes (*APE1*, *hOGG1*, *XRCC1*, *XRCC3*, *XPD*, *PARP1*, *MGMT* genes; representative of the different DNA repair pathways operating in mammals) in 77 hospital workers chronically exposed to low doses of IR, and 70 matched controls. A significantly higher MNi frequency was found in the exposed group (16.2±10.4 *vs.* 11.5±9.4; *P*=0.003) and the effect appeared to be independent from the principal confounding factor. Exposed individuals with *hOGG1*, *XRCC1*, *PARP1*, and *MGMT* wild-type alleles or *APEX1*, as well as *XPD* (rs13181) heterozygous showed a significantly higher MNi frequency than controls with the same genotypes. Genetic polymorphism analysis and cytogenetic dosimetry have proven to be a powerful tool complementary to physical dosimetry in regular health surveillance programmes.

KEY WORDS: *genotype analysis; micronucleus; nuclear bud; nucleoplasmic bridge; occupational exposure*

The health consequences of continuous exposure to low doses of ionising radiation (IR) are still a topic of great scientific interest. Medical workers are the most commonly studied group among chronically exposed professionals, with regular medical surveillance and obligatory dosimetry. The duration and amount of received radiation have significantly decreased over the last decades, with received doses well below the allowable limits of 20 mSv per year. However, most studies on occupationally exposed subjects have shown an increase in genetic damage after chronic exposure to IR low doses, without evidence of any dose-effect relationship. Nevertheless, recent literature, including a paper on as many as 400.000 nuclear power plant workers, shows significant correlation between accumulated doses and risk of tumour development (1-4).

The cytokinesis-blocked micronucleus (CBMN) assay has been widely used to evaluate DNA damage after occupational, therapeutic or accidental exposure to IR, as well as to assess *in vitro* radiosensitivity and cancer susceptibility. For instance, in Belgium and Croatia, the CBMN assay is regularly applied in the biomonitoring of workers exposed to IR higher than or expected to reach 20 mSv (5). In recent years, the CBMN assay has evolved into the novel cytome assay, where every cell is scored for its damage and mitotic status (6). Originally, the CBMN assay was developed to measure micronuclei (MNi) - whole chromosomes or acentric chromosome fragments that lag behind during anaphase and are not distributed to the main nuclei. Subsequently it was observed that the CBMN assay may also measure other forms of damage, such as nuclear buds (NBs) and nucleoplasmic bridges (NPBs). NBs have been proposed as markers of gene amplification and/or altered gene dosage; NPBs provide a measure of chromosome rearrangement, or DNA misrepair, and may break to form MNi (6). The CBMN assay also allows one to calculate the nuclear division index (NDI), providing information on a cell cycle's delay with regard to exposure.

Individual sensitivity to IR exposure is the result of a close interaction between DNA damage and DNA repair. Several authors have already described the association between genetic damage, IR exposure, and polymorphisms in DNA repair genes (7-10).

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To the best of our knowledge, this is the first study to evaluate if biomarkers of the CBMN cytome assay (MNi, NPBs, NBs, and NDI) could be sensitive enough to evaluate the impact of occupational exposure to IR low doses. Moreover, we investigated the possible influence on biomarkers of the CBMN cytome assay of a comprehensive panel of polymorphisms in DNA repair genes. Included in the analysis were polymorphisms in the *APE1*, *hOGG1*, *XRCC1*, *XRCC3*, *XPD*, *PARP1*, and *MGMT* genes, representative of the different DNA repair pathways operating in mammals.

MATERIALS AND METHODS

Study population

The study included 77 medical workers occupationally exposed to low doses of IR, and 70 controls who had never been occupationally exposed to IR or other known carcinogenic agents. All of the subjects gave their written consent after being informed on the study scope and experimental details. The study followed the guidelines of the Declaration of Helsinki regarding medical research involving human subjects, and was approved by an ethics committee. Standardised questionnaires were administered to all of the participants to determine their socio-demographic characteristics, medical history (e.g. history of medical treatments, radiography, recent vaccination, severe infections, or viral diseases over the past six months, presence of known inherited genetic disorders and chronic diseases, family history of cancer), and individual life styles [e.g. smoking, alcohol consumption, dietary habits, including deficient or peculiar habits (e.g. vegetarian or vegans), or intake of multi-vitamins supplements, and use of contraceptive]. Exclusion criteria included the use of any therapeutic drugs, radiotherapy, diagnostic X-rays undergone 12 months prior to sampling, which could have significantly contributed to the received dose and/or genetic damage. For medical workers, the questionnaires covered the duration of occupational exposure to IR. Selected demographic characteristics of the study population are reported in Table 1. Among the medical workers, we distinguished between seven different working tasks: gastroenterologist, interventional cardiologist, anaesthesiologists, surgeons, radiologists, and engineers of radiology. All of the IR exposed workers were under medical surveillance and regular film dosimetry, however according to the written consent provide to the subjects, we only know the annual dose received did not exceed the limit of 20 mSv per year.

Venous blood was obtained from each subject and transferred to the laboratory within a few hours for subsequent CBMN assay and genotype analysis.

Table 1 *Demographic characteristics of the study population*

	Exposed	Controls	Total
Sample size (n)	77	70	147
Sex			
Female n (%)	46 (59.7)	26 (37.1)	72 (49.0)
Male n (%)	31 (40.3)	44 (62.9)	75 (51.0)
Age			
mean±SD	42.2 ± 10.6	40.8 ± 10.4	41.5 ± 10.5
range	23-69	20-60	20-69
Smoking status			
Never (%)	47 (61.0)	50 (71.4)	97 (66.0)
Current (%)	30 (39.0)	20 (28.6)	50 (34.0)
Years of exposure			
mean±SD	13.7±8.9	-	-
range	1-38		

**Significantly lower compared to controls* P*=0.032 (Wilcoxon test)*

CBMN cytome assay

Cultures for the CBMN assay were set up in triplicate. Lymphocytes were cultured in RPMI 1640 medium (Gibco, Paisley, UK), supplemented with 1 % of phytohaemagglutinin, (Apogent, USA), 20 % of foetal calf serum (Gibco), and 1 % penicillin-streptomycin solution (Sigma-Aldrich, St Louis, MO, USA) and incubated at 37 °C in humidified 5 % CO_2 atmosphere for 72 h. After 44 h, all cultures were supplemented with cytochalasin B (Sigma; final concentration 6 μg mL^{-1}). At the end of the incubation period, lymphocytes were subjected to a mild hypotonic treatment**,** fixed, and stained according to Kapka et al. (11). One thousand binucleated lymphocytes with well-preserved cytoplasm per subject were analysed. The criteria for analysis of MNi, NPBs, and NBs were as described by Fenech et al. (12). NDI was calculated according to the formula NDI=$(1M_1+2M_2+3M_3+4M_4)/1000$ cells where M_1-M_4 indicates lymphocytes with 1 to 4 nuclei (13).

DNA isolation

Genomic DNA was isolated from EDTA-anticoagulated whole blood using standard sodium perchlorate/chloroform extraction procedures or the QIAamp DNA Blood kit (Qiagen, Hilden, Germany) as recommended by the manufacturer. For genotype analysis, DNA samples were diluted and stored as 10 ng μL^{-1} aliquots at -20 °C. Genotyping was performed by PCR-based assays: RFLP and/or real-time (Table 2) (9, 14, 15). Negative controls were included in each reaction as quality control. Genotyping by real-time PCR was performed by the 5′-nuclease allelic discrimination assay (TaqMan®, Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instruction. Genotype screening was carried out simultaneously in a blinded manner to work allocation (exposed, non-exposed). Genotype results were regularly confirmed by repetition of 90 % of the samples.

Table 2 *Details on investigated SNPs in DNA repair genes*

GENE rs unique code [base and amino acid change]	METHOD[A]
APEX1	
rs1130409 [gaT > gaG; Asp148Glu]	RFLP [Hu et al., 2002] (14) RT TaqMan assay C_8921503_10
hOGG1	
rs1052133 [tCc > tGc; Ser326Cys]	RFLP [Godderis et al., 2006] (15) RT TaqMan assay C_3095552_1
XPD	
rs1799793 [Gac > Aag; Asp312Asn] rs13181 [Aag > Cag; Lys751Gln]	RT TaqMan assay C_3145050_10 RFLP [Angelini et al., 2005] (8) RT TaqMan assay C_3145033_10
XRCC1	
rs861539 [cAg > cGg; Gln399Arg]	RFLP [Angelini et al., 2005] (8) RT TaqMan assay C_622564_10
XRCC3	
rs861539 [aCg > aTg; Thr241Met]	RFLP [Angelini et al., 2005] (8) RT TaqMan assay C_3145033_10
PARP1	
rs1136410 [gTg > gCg; Val762Gly]	RT TaqMan assay C_1515368_1_
MGMT	
rs12917 [Ctt > Ttt; Leu115Phe]	RT TaqMan assay C_3157955_10

[A]RFLP=PCR-RFLP analysis carried out according to published methods (reference parenthetically); RT=Real-Time PCR with TaqMan allelic discrimination assay (Applera, Foster City, USA)

Statistical analysis

The Wilcoxon rank-sum test was used to test the difference in MNi, NB, and NPB frequency between exposed workers and controls. The association between MNi, NB, and NPB frequency and the various genotypes was tested by Kruskal-Wallis test. The distribution of genotypes was tested for Hardy-Weinberg (HW) equilibrium using the online HW test tool offered by the Institute for Human Genetics, Technical University Munich, Germany. Linear regression analysis was applied to assess the correlation between years of IR exposure and MNi, NBs, and NPBs in the exposed workers. The Poisson regression analysis was applied to evaluate the influence of age, sex, smoking status, and working task on MNi, NPBs, and NBs in the overall population and in both groups separately. The level of significance was set at $P<0.05$; statistical analysis was conducted using Stata Intercooled version 11.0 (16).

RESULTS

The principal demographic characteristics of the study population, both overall and by group are reported in Table 1. In summary, age distribution was similar in the two groups ($P=0.212$); while sex was significantly different, as females were over-represented in the IR-exposed individuals than controls (59.7 *vs*. 37.1 %, $P=0.006$). Regarding smoking status, 39.0 % of the exposed workers and 28.6 % controls were smokers at the time of sampling. No difference was observed between the two groups in terms of years of smoking or daily cigarette consumption, all being mild smokers (less than 10 cigarettes per day).

CBMN assay

Results of CBMN cytome assay (MNi, NPB, NB frequencies and NDI) are presented in Table 3. Of the four analysed parameters, only MNi frequency was significantly higher in the exposed workers than in controls ($P=0.003$). The frequencies of the other parameters were similar between the exposed and controls. The range of IR exposure duration in radiological workers, i.e. years of employment, was 1-38 (Table 1). Linear regression analysis revealed a significant association between years of employment and MNi ($\beta=0.403$, $P=0.003$; Figure 1A) and NBs ($\beta=0.075$, $P=0.027$; Figure 1B), whereas no association emerged with NPBs ($\beta=0.024$, $P=0.230$).

The results of Poisson regression analysis reporting the influence of confounding factors, which included age, sex, and smoking status on MNi, NPBs, and NBs, are reported in Table 3 to Table 6 respectively. With regard to MNi (Table 4), an increase in age resulted in a significant increase in MNi frequency both in controls and exposed workers ($P<0.0001$ for both). Sex exerted a significant influence on the yield of MNi only in controls, with the frequency being

Table 3 *Results of micronucleus assay in the study groups*

	Exposed	Controls
Micronuclei		
Mean±SD	16.2±10.4*	11.5±9.4
(Range)	1-47	0-37
Nucleoplasmatic bridges		
Mean±SD	0.9±1.5	1.7±4.0
(Range)	0-8	0-30
Nuclear buds		
Mean±SD	1.7±2.6	2.2±3.5
(Range)	0-15	0-23
Nuclear division index		
Mean±SD	1.8±0.3	1.9±0.4
(Range)	1.2-2.3	0-2.7

**Significantly higher compared to controls* P=*0.003 (Wilcoxon test)*

higher in females as compared to males (*P*=0.002). Smoking status significantly influenced MNi frequency in both study groups, however at an opposite trend: being higher in current smokers compared to non-smokers in the IR exposed group, whereas in controls current smokers were characterized by lower MNi frequency than those that had never smoked (*P*<0.0001 for both). Regarding NPB frequency (Table 5), only sex exerted a significant influence in both study groups. In particular, NPB frequency was significantly higher in males compared to females (*P*=0.004 in IR exposed; *P*=0.015 in controls). In the control group, we also observed a significant influence of age (*P*<0.0001). As regard to NBs (Table 6), in the IR exposed group we observed a significant age-dependent effect (*P*=0.001). Non-smokers were characterised by a significantly higher NB frequency as compared to current smokers (*P*=0.002). In the control group, we observed the influence of sex, with NBs being significantly higher in males compared to females (*P*=0.008), and a significant age-dependent effect (*P*=0.008).

Poisson regression analysis applied to the overall study population highlighted the significant influence of age on MNi, NPBs, and NBs. In all three instances, an increase of age was associated with a significant increase of the observed frequency of DNA damage (*P*<0.0001 for all). With regard to sex, MNi frequency was lower in males, although not significantly (*P*=0.064), whereas the opposite results were observed for NPB and NB frequency, being significantly higher in males than female (*P*<0.0001 and *P*=0.013 respectively). We observed a significant effect of smoking status only in NBs; surprisingly this biomarker was higher in non-smokers compared to active smokers (*P*<0.0001). Interestingly, when dividing smokers into two classes (0-10 and >10 years) of smoking habits - a significant effect was observed only in the second group. In particular, individuals who smoked for more than 10 years exhibited a significantly lower NBs frequency (IRR 0.579, 95 % CI 0.403-0.833; *P*=0.003), whereas no effect was observed in those who smoked less than 10 years.

Table 4 *Poisson regression analysis of confounding factors on MNi frequencies*

Confounding factors[a]	IRR	*P*	95 % CI
All			
Age (years)	1.020	<0.0001	1.016-1.025
Sex (0,1)	0.918	0.064	0.839-1.004
Smoking status (0,1)	1.047	0.335	0.954-1.149
Exposure (years)	1.332	<0.0001	1.214-1.460
Controls			
Age (years)	1.018	<0.0001	1.011-1.026
Sex (0,1)	0.801	0.002	0.696-0.923
Smoking status (0,1)	0.729	<0.0001	0.612-0.867
Exposed workers			
Age (years)	1.020	<0.0001	1.015-1.026
Sex (0,1)	0.971	0.621	0.864-1.091
Smoking status (0,1)	1.227	<0.0001	1.094-1.376

IRR: Incidence Rate Ratio
[a]Sex: 0-Female, 1-Male; Smoking status: 0-Never, 1-Current

Table 5 *Poisson regression analysis of confounding factors on NPBs frequencies*

Confounding factors[a]	IRR	*P*	95 % CI
All			
Age (years)	1.031	<0.0001	1.016-1.046
Sex (0,1)	1.771	<0.0001	1.290-2.433
Smoking status (0,1)	0.863	0.377	0.623-1.196
Exposure (years)	0.621	0.002	0.458-0.843
Controls			
Age (years)	1.049	<0.0001	1.029-1.070
Sex (0,1)	1.700	0.015	1.111-2.603
Smoking status (0,1)	1.013	0.951	0.660-1.557
Exposed workers			
Age (years)	1.008	0.457	0.987-1.030
Sex (0,1)	2.021	0.004	1.258-3.246
Smoking status (0,1)	0.718	0.189	0.439-1.176

IRR: Incidence Rate Ratio
[a]Sex: 0-Female, 1-Male; Smoking status: 0-Never, 1-Current

Table 6 *Poisson regression analysis of confounding factors on NBs frequencies*

Confounding factors[a]	IRR	*P*	95 % CI
All			
Age (years)	1.025	<0.0001	1.013-1.037
Sex (0,1)	1.368	0.013	1.069-1.751
Smoking status (0,1)	0.597	<0.0001	0.448-0.795
Exposure (years)	0.841	0.164	0.660-1.073
Controls			
Age (years)	1.023	0.008	1.006-1.040
Sex (0,1)	1.635	0.008	1.136-2.353
Smoking status (0,1)	0.703	0.088	0.469-1.054
Exposed workers			
Age (years)	1.029	0.001	1.012-1.047
Sex (0,1)	1.166	0.396	0.817-1.663
Smoking status (0,1)	0.530	0.002	0.354-0.795

IRR: Incidence Rate Ratio
[a]Sex: 0-Female, 1-Male; Smoking status: 0-Never, 1-Current

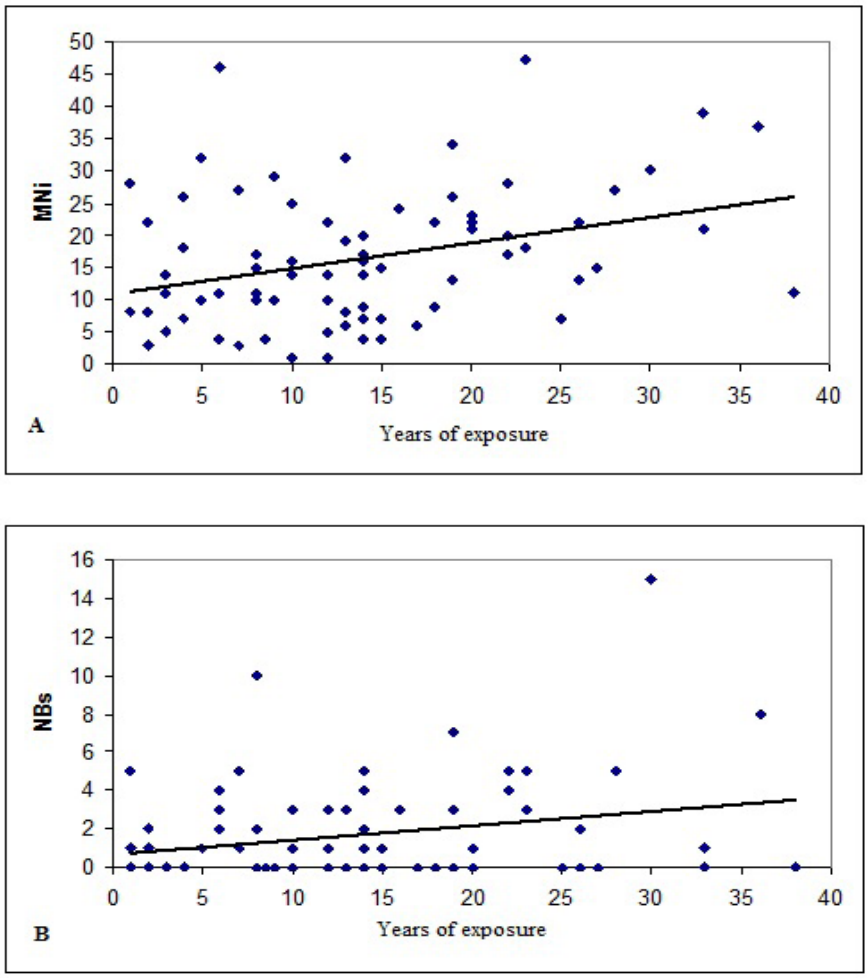

Figure 1 *Relationship between DNA damage, assessed as micronuclei [MNi (A)] and nuclear buds [NBs (B)] in peripheral blood lymphocytes, and years of exposure to ionising radiation. The thick line represents the linear regression analysis of the data*

With regard to exposure, Poisson analysis revealed that an increase in the years of IR exposure was associated with a significant increase in MNi frequency ($P<0.0001$) and surprisingly a significant decrease of NPBs ($P=0.002$). No effect of years of exposure on NBs was observed.

Genotype distribution and influence on biomarkers of the cytome assay

Details on the investigated SNPs in DNA repair genes are represented in Table 2. Genotype distribution of the eight studied DNA repair genes among the different study groups and in the overall population is presented in Table 7. Frequencies of the variant allele observed in our study were consistent with those reported in the publicly available database NCBI (dbSNP) for Caucasians. Deviation from the HW equilibrium was observed for one SNP in the IR exposed group (*XPD* rs13181); departure from HW equilibrium was not observed for any other SNPs in any of the studied group, or in the overall population. Allele frequencies were similar between the IR exposed and controls, with the exception of *hOGG1* rs1052133 and *PARP1* rs1136410 SNPs. To be more precise, the *hOGG1* variant allele and *PARP1* wild-type allele were significantly underrepresented in controls compared to IR exposed ($P=0.0017$, and $P=0.039$ respectively).

Distribution of MNi, NPBs and NBs by DNA repair genotypes and exposure status are shown in Table 8 to Table 10 respectively. For several genes, due to the small number of individuals homozygous for the variant allele, the approach was to group them together with heterozygous in order to increase statistical power. Among the controls, *PARP1* wild type allele was associated with significantly higher NBs compared to the combined homozygous SNP plus heterozygous genotype (2.5±3.0 *vs.* 1.0±1.4, $P=0.044$; Table 9). In the IR exposed group, the same genotype was also significantly associated with higher NPBs (1.2±1.7 *vs.* 0.4±0.8, $P=0.027$; Table 10). No detectable influence of other genotypes on MNi, NBs or NPBs was observed within the two groups. When we compared IR exposed workers with controls, a significantly higher MNi frequency was found in radiological workers homozygous wild-type for the *hOGG1*, *XRCC1*, *XRCC3*, *MGMT1*, and *PARP1* compared to controls with the same genotypes (Table 8).

Table 7 *Genotype distribution, allele frequency and Hardy-Weinberg equilibrium of the investigated SNPs in DNA repair genes*

Genotype*	Exposed (n=77)	Controls (n=56)**	Total (n=133)
APEX1 rs1130409			
0	26 (33.8)	19 (33.9)	45 (33.8)
1	35 (45.5)	27 (48.2)	62 (46.6)
2	16 (20.7)	10 (17.9)	26 (19.6)
	q=0.44; P_{HWE}=0.509	q=0.42; P_{HWE}=0.939	q=0.43; P_{HWE}=0.578
		Pafd=0.802	
hOGG1 rs1052133			
0	38 (49.3)	42 (75.0)	80 (60.1)
1	34 (44.1)	14 (25.0)	48 (36.1)
2	5 (6.6)	0 (0.0)	5 (3.8)
	q=0.29; P_{HWE}=0.285	q=0.12; P_{HWE}=0.473	q=0.22; P_{HWE}=0.501
		Pafd=0.0017	
XPD rs1799793			
0	31 (40.3)	17 (30.4)	48 (36.1)
1	33 (42.8)	33 (58.9)	66 (49.6)
2	13 (16.9)	6 (10.7)	19 (14.3)
	q=0.38; P_{HWE}=0.413	q=0.40; P_{HWE}=0.091	q=0.39; P_{HWE}=0.628
		Pafd=0.758	
XPD rs13181			
0	32 (41.6)	15 (27.2)	47 (35.3)
1	27 (35.1)	33 (58.9)	60 (45.1)
2	18 (23.3)	8 (13.9)	26 (19.6)
	q=0.41; P_{HWE}=0.016	q=0.44; P_{HWE}=0.140	q=0.42; P_{HWE}=0.389
		Pafd=0.643	
XRCC1 rs861539			
0	31 (40.2)	22 (39.3)	53 (39.9)
1	41 (53.2)	25 (44.6)	66 (49.6)
2	5 (6.6)	9 (16.1)	14 (10.5)
	q=0.33; P_{HWE}=0.076	q=0.38; P_{HWE}=0.674	q=0.35; P_{HWE}=0.322
		Pafd=0.374	
XRCC3 rs861539			
0	23 (29.9)	23 (42.6)	46 (35.1)
1	38 (49.3)	25 (46.3)	63 (48.1)
2	16 (20.8)	6 (11.1)	22 (16.8)
	q=0.45; P_{HWE}=0.967	q=0.34; P_{HWE}=0.838	q=0.41; P_{HWE}=0.956
		Pafd=0.070	
PARP1 rs1136410			
0	55 (71.4)	33 (58.9)	88 (66.2)
1	21 (27.3)	18 (32.1)	39 (29.3)
2	1 (1.3)	5 (9.0)	6 (4.5)
	q=0.15; P_{HWE}=0.520	q=0.25; P_{HWE}=0.285	q=0.19; P_{HWE}=0.534
		Pafd=0.039	
MGMT rs12917			
0	56 (72.7)	39 (69.6)	95 (71.4)
1	18 (23.4)	16 (28.6)	34 (25.6)
2	3 (3.9)	1 (1.8)	4 (3.0)
	q=0.16; P_{HWE}=0.328	q=0.16; P_{HWE}=0.658	q=0.16; P_{HWE}=0.655
		Pafd=0.914	

**0=homozygous wild-type (wt); 1=Heterozygous (HE); 2=homozygous polymorphic (SNP)*
q=frequency of the SNP
P_{HWE}=P-*value of the Hardy-Weinberg Equilibrium*
Pafd=P-*value of the allele frequency difference between controls and IR exposed*
***14 samples are missing due to lack of biological material*

Table 8 *Mean MNi frequencies in the study population subdivided by exposure status and genotype distribution*

	Exposed (n=77)			Controls (n=56)		
Gene*	**Subject (n)**	**Mean±SD**	**95 % CI**	**Subject (n)**	**Mean±SD**	**95 % CI**
APEX1 rs1130409						
0	26	15.1±10.2	11.0 -19.2	19	11.0±10.3	6.0-16.0
1	35	17.3±11.2[a]	13.5-21.1	27	10.3±8.8	6.8-13.8
2	16	15.5±9.5	10.4-20.6	10	12.2±7.1	7.1-17.3
hOGG1 rs1052133						
0	38	15.8±10.2 [b]	12.4-19.1	42	10.6±9.2	7.7-13.4
1+2	39	16.6±10.8	13.1-20.1	14	11.7±8.6	6.7-16.7
XPD rs1799793						
0	31	16.5±11.1	12.4-20.6	17	11.4±7.3	7.7-15.2
1+2	46	16.0±10.1 [c]	16.0-19.0	39	10.6±9.7	7.5-13.8
XPD rs13181						
0	32	16.7±10.8	12.8-20.6	15	11.6±7.7	7.3-15.8
1	27	16.4±10.7 [d]	12.1-20.6	33	9.8±8.5	6.7-12.8
2	18	15.0±9.8	10.1-19.9	8	14.0±12.8	3.3-24.7
XRCC1 rs861539						
0	31	17.3±12.3 [e]	12.8-21.8	22	9.6±8.8	5.7-13.5
1+2	46	15.4±9.0	12.8±18.1	34	11.6±9.1	8.5-14.8
XRCC3 rs861539						
0	23	18.3±9.9 [f]	14.0-22.6	23	11.8±9.8	7.6-16.0
1+2	54	15.3±10.6 [g]	12.4-18.2	31	9.7±8.4	6.6-12.7
PARP1 rs1136410						
0	55	17.3±10.5 [h]	14.4-20.1	33	11.3±8.9	8.2-14.5
1+2	22	13.5±9.9	9.1-17.9	23	10.4±8.8	6.2-14.2
MGMT rs12917						
0	56	18.2±10.6 [i]	15.3-21.0	39	11.1±9.2	8.1-14.1
1+2	21	11.0±8.0	7-3-14.6	17	10.2±8.7	5.8-14.7

**0=homozygous wild-type (wt); 1=Heterozygous (HE); 2=homozygous polymorphic (single nucleotid polymorphism, SNP)*
Significantly different from controls with the same genotypes (Wilcoxon test): [a]P=0.005; [b]P=0.012; [c]P=0.004; [d]P=0.003; [e]P=0.010; [f]P=0.017; [g]P=0.007; [h]P=0.008; [i]P=0.001

With regard to *XRCC3*, MNi frequency was also significantly higher in IR exposed homozygous SNP pooled with heterozygous, compared to controls with the same pooled genotype. In addition, MNi frequency was also significantly higher in IR exposed heterozygous for *APEX1* and *XPD* rs13181. Regarding *XPD* rs1799793, we also observed a significantly higher MNi frequency in homozygous SNP pooled with heterozygous, compared to controls with the same pooled genotype. Concerning the *APEX1* genotype, we also observed an influence on NB frequency. In particular, significantly lower NBs frequency was observed in IR exposed workers homozygous SNP compared to controls with the same genotype (Table 9).

DISCUSSION

CBMN assay has already shown to be a reliable biomarker in the evaluation of IR exposure in different settings, including radiotherapy and occupational and accidental environmental exposure (17). The strength and novelty of the present study lies in its investigation of biomarkers of the CBMN cytome assay, which includes NPBs and NBs in addition to MNi. To the best of our knowledge, this is the first study to analyse NPBs and NBs in individuals occupationally exposed to IR-low doses. The analysis of NPBs and NBs in lymphocytes has become increasingly important for their sensitivity in revealing chromosomal damage in humans. The results of our study showed that MNi frequency was significantly higher in the IR exposed workers compared to controls. The significance was unlikely to be related to differences in age, sex, and smoking within the two groups, as the multivariate analysis showed that MNi frequency was significantly influenced by IR exposure. This finding confirms the reliability of the MNi frequency as biological dosimetry in population occupationally exposed to low doses of IR, as shown in several earlier cytogenetic studies (9, 10, 18-23). To date, none of the studies on IR occupationally exposed workers have included the analysis of NPBs and NBs. Surprisingly, in our study these biomarkers were higher in controls compared to IR exposed workers, however the differences were small and statistically not significant. None of the occupationally exposed subjects studied here had ever exceeded the permitted radiation limit for occupational exposure, recommended by the International Commission on Radiological Protection (ICRP). The lack of knowledge of the IR dose equivalent to the whole body (external whole-body dose equivalent, *Hwb*), accumulated over the entire working-life period may represent a limitation of the present study. On the other hand, most studies to date have failed

Table 9 *Mean NB frequencies in the study population subdivided by exposure status and genotype distribution*

	Exposed (n=77)			Controls (n=56)		
Gene*	**Subject (n)**	**Mean±SD**	**95 % CI**	**Subject (n)**	**Mean±SD**	**95 % CI**
APEX1 rs1130409						
0	26	1.5±2.0	0.7-2.3	19	1.2±1.9	0.3-2.1
1	35	1.9±2.5	1.1-2.8	27	2.0±2.6	1.0-3.1
2	16	1.6±3.8 [a]	0.6-3.8	10	3.0±3.1	0.8-5.2
hOGG1 rs1052133						
0	38	1.4±2.3	0.7-2.2	42	1.8±2.6	1.0-2.6
1+2	39	1.9±2.9	1.0-2.9	14	2.3±2.5	0.8-3.7
XPD rs1799793						
0	31	1.7±1.7	1.1-2.3	17	3.0±3.4	1.2-4.8
1+2	46	1.7±3.1	0.8-2.6	39	1.4±1.9	0.8-2.0
XPD rs13181						
0	32	1.9±1.7	1.2-25	15	2.6±3.8	0.5-4.7
1	27	1.6±3.3	0.3-2.9	33	1.7±1.8	1.0-2.3
2	18	1.5±3.0	0.1-3.0	8	1.6±2.1	0.1-3.4
XRCC1 rs861539						
0	31	1.3±1.9	0.6-2.0	22	2.1±3.2	0.7-3.5
1+2	46	2.0±3.0	0.7-1.8	34	1.8±2.0	1.1-2.5
XRCC3 rs861539						
0	23	1.6±3.2	0.2-3.0	23	2.0±2.3	1.0-2.9
1+2	54	1.8±2.4	1.1-2.4	31	2.0±2.8	0.9-3.0
PARP1 rs1136410						
0	55	1.9±2.8	1.1-2.7	33	2.5±3.0	1.5-3.6
1+2	22	1.2±2.0	0.4-2.1	23	1.0±1.4[b]	0.5-1.6
MGMT rs12917						
0	56	1.9±2.9	1.1-2.7	39	1.7±2.4	0.9-2.5
1+2	21	1.2±1.6	0.5-2.0	17	2.4±2.9	0.3-2.8

**0=homozygous wild-type (wt); 1=Heterozygous (HE); 2=homozygous polymorphic (SNP)*
[a]Significantly different from controls with the same genotypes (Wilcoxon test, P*=0.041)*
[b]Significantly different from homozygous wild-type controls (Kruskal-Wallis test, P*=0.044)*

Table 10 *Mean NPB frequencies in the study population subdivided by exposure status and genotype distribution*

	Exposed (n=77)			Controls (n=56)		
Gene*	**Subject (n)**	**Mean±SD**	**95 % CI**	**Subject (n)**	**Mean±SD**	**95 % CI**
APEX1 rs1130409						
0	26	1.0±1.8	0.3-1.7	19	0.7±1.4	0.1-1.4
1	35	1.0±1.4	0.5-1.4	27	1.7±2.2	0.8-2.5
2	16	0.8±1.5	0.1-1.6	10	1.8±2.3	0.1-3.5
hOGG1 rs1052133						
0	38	0.7±1.1	0.4-1.1	42	1.1±1.8	0.6-1.7
1+2	39	1.2±1.9	0.6-1.8	14	2.1±2.6	0.6-3.6
XPD rs1799793						
0	31	0.7±1.0	0.3-1.1	17	1.4±2.3	0.2-2.6
1+2	46	1.1±1.8	0.6-1.7	39	1.4±1.9	0.7-2.0
XPD rs13181						
0	32	1.0±1.8	0.4-1.7	15	1.5±2.4	0.1-2.8
1	27	0.6±0.9	0.3-1.0	33	1.3±1.8	0.7-1.9
2	18	1.3±1.8	0.4-2.2	8	1.5±2.5	0.1-3.5
XRCC1 rs861539						
0	31	0.7±1.0	0.3-2.1	22	1.6±2.6	0.5-2.8
1+2	46	1.1±1.8	0.6-1.6	34	1.2±1.6	0.7-1.8
XRCC3 rs861539						
0	23	1.3±2.0	0.4-2.1	23	1.5±2.3	0.5-2.5
1+2	54	0.8±1.2	0.5-1.2	31	1.3±1.9	0.6-2.0
PARP1 rs1136410						
0	55	1.2±1.7	0.7-1.6	33	1.8±2.5	1.0-2.7
1+2	22	0.4±0.8[a]	0.1-0.8	23	0.7±0.8	0.4-1.1
MGMT rs12917						
0	56	1.1±1.5	0.6-1.5	39	1.3±1.9	0.7-1.9
1+2	21	0.7±1.5	0.1-1.3	17	1.5±2.4	0.3-2.8

**0=homozygous wild-type (wt); 1=Heterozygous (HE); 2=homozygous polymorphic (SNP)*
[a]Significantly different from homozygous wild-type IR exposed (Kruskal-Wallis test, P*=0.027)*

to find any association between chromosome damage and *Hwb* accumulated after chronic exposure to low doses (9, 19, 20, 22, 24, 25). From this point of view, the years the workers were occupationally exposed to IR may represent an attempt to overcome the limits associated with the use of dosimeters. Positive correlation of MNi frequency and exposure duration has been confirmed in previous studies (23, 26), with a reported elevated high risk of cancer in medical X-ray workers as their service duration increased (2). Our IR exposed group demonstrated a clear relation between MNi and NB frequencies, that is, an increase of 0.4 and 0.07 in the number of MNi and NBs respectively, per 1 year of IR occupational exposure.

Age, sex, and smoking status are common confounding factors known to affect MNi frequency, whereas information on NBs and NPBs are limited. Despite the relatively small sample size, our data confirm the effect of aging on MNi frequency in all of the studied groups – i.e. exposed, controls and overall population. The same age dependent effect was seen for NBs, a finding that might not be surprising as the nuclear budding process has emerged as another unique mechanisms of MNi formation. NBs are also associated with the alteration of DNA stability, with evidence that these structures contain entire or fragments of chromosomes (6, 27). Our study is in agreement with an international collaborative study on pooled data from 25 laboratories that confirmed the impact of sex on MNi frequencies among subjects involved in occupational and environmental surveys (28), although in our case female subjects differed significantly from the male only in the control group. In the entire studied population, men had a higher NPB frequency, as in another study on healthy volunteers (29), but the knowledge of the effect of confounding factor on NPBs frequencies is not as extensive as on MNi. Generally, the effects of smoking on DNA damage in individuals exposed to IR are still unclear, with opposite results (9, 20, 24, 26, 30, 31). In IR exposed subjects, smoking habits were associated with increased MNi frequency, which is in agreement with previous studies (20, 30), but there was a significantly decreased MNi frequency in the control group. Interestingly, NB frequency was significantly decreased both in the IR exposed workers and in the overall population. In our study population, none of the individuals reported being heavy smokers (≥30 cigarettes per day), which made it impossible to further study the potential increase of genetic damage, primarily MNi. It has been shown that MNi frequency among occupationally and environmentally exposed individuals is influenced only in non-exposed heavy smokers (32) and slightly reduced in smokers exposed to genotoxic agents, with two possible explanations: appearance of apoptotic/necrotic cells due to cigarette smoke damage that would not be detected in the CBMN assay (20,29) and possible adaptive response stimulation caused by the intake of a few cigarettes per day, causing a lowering in MN frequency (29).

Another critical consideration is the cellular response to IR low doses. Exposure is a complex mechanism, leading to the activation of multiple signal transduction pathways, which besides DNA repair include apoptosis, proliferation, inflammation, and genomic instability. Therefore, different genes, belonging to these different pathways, and characterised by several polymorphisms, may contribute to the individual genome sensitivity in IR exposed subjects (8-10, 33-36). Genotype analysis revealed an association between all of the investigated polymorphisms and MNi frequency, while only the *APEX1* polymorphism was associated with NB frequency. With regard to the *XRCC3* polymorphism, the effect on MNi is unclear. Both genotype groups in the IR exposed group had significantly higher MNi frequencies compared to controls with the same genotypes, with no difference within the group. This hypothesis corroborates *in vitro* studies that reported an association between increased sensitivity to IR in human lymphocytes and SNPs in DNA repair genes (7, 36). It is feasible that gene-gene interactions may influence DNA damage in response to dose; however, the reduction in sample sizes, as a consequence of successive categorization, even further limits the already weak statistical power (due to small sample size). It is undoubtedly possible that some of the associations become significant by chance, due to the inadequate statistical power. However, this does not rule out an association between DNA repair gene polymorphisms and DNA damage frequency (36). Achieving a complete understanding of this interaction may be of great importance for implementing radiation protection and radiotherapy programmes (37, 38). In view of this, concomitant analysis of DNA damage and interindividual differences in DNA repair genes, due to the presence of polymorphisms, may represent a valuable multi-biomarker approach (39).

In conclusion, our group was the first to score MNi, NBs, and NPBs in IR exposed workers. Clearly, the results obtained confirmed the genotoxic implication resulting from the occupational exposure to IR low doses. Moreover, we believe we have confirmed the value of the MNi frequency as a standard and powerful cytogenetic method for studying genotoxicity in populations exposed to IR low doses. Whether the cytome assay, meaning NBs and NPBs that were not found to be higher, could improve the predictive capacity of the assay to reveal genetic damage after chronic IR low dose exposure, remains to be determined. Obviously, before ruling out the usefulness of NB and NPB analysis in IR exposed individuals, it would be desirable to replicate the study on a larger population. Nevertheless, it might be interesting to apply the cytome assay in populations exhibiting different IR exposure levels. In particular, interventional cardiologists are currently exposed to a significantly radiation risk compared to other occupational IR exposed individuals.

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Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Polimorfizmi u genima za popravak DNA: poveznica s biomarkerima mikronukleus-testa u medicinskih radnika kronično izloženih niskim dozama ionizirajućeg zračenja

Individualna osjetljivost na ionizirajuće zračenje rezultat je međudjelovanja samog izlaganja zračenju, oštećenja DNA nastalog prilikom tog izlaganja te samog popravka nastalog oštećenja. Veliki doprinos razlikama čine i polimorfizmi u genima za popravak DNA. U ovom radu istražili smo povezanost nastalih oštećenja DNA u obliku mikronukleusa (MN), jezgrinih pupova (NB) i nukleoplazmatskih mostova (NPB) s polimorfizmima jednog nukleotida (SNP) u genima za popravak DNK (*APE1, hOGG1, XRCC1, XRCC3, XPD, PARP1, MGMT*) koji sudjeluju u različitim mehanizmima popravka. Rezultati skupine od 77 medicinskih radnika kronično izloženih niskim dozama ionizirajućeg zračenja uspoređeni su s rezultatima skupine od 70 odgovarajućih kontrola. Izložena skupina imala je značajno veću učestalost MN-a (16,2±10,4 *vs.* 11.5±9.4; *P*=0,003), a sama pojavnost oštećenja bila je neovisna o medijatornoj varijabli (kovarijati). Značajno više učestalosti MN nađene su u izloženoj skupini u homozigotnih nositelja divljeg tipa gena *hOGG1, XRCC1, PARP1* i *MGMT* i u heterozigotnih nositelja gena *APEX1* i *XPD* (rs13181) u odnosu na kontrolnu skupinu istoga genotipa. Analiza genskih polimorfizama i citogenetička dozimetrija važna su dopuna osobnom dozimetrijskom nadzoru izloženih radnika.

KLJUČNE RIJEČI: *genotipska analiza; jezgrini pupovi; mikronukleus; nukleoplazmatski mostovi; profesionalna izloženost*