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Correlation between Micronuclei Frequency in Peripheral Blood Lymphocytes and Retention of 131-I in Thyroid Cancer Patients

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Differentiated thyroid cancers (DTCs) derive from thyroid follicular cells and include papillary and follicular cancers. In patients with DTCs, the initial treatment includes thyroidectomy and radioactive iodine (131-I) therapy. The objective of this study was to examine whether the intensity of DNA damage in peripheral blood lymphocytes (PBLs) of DTC patients depends on the amount of 131-I retained in the selected regions of interest (thyroid and abdominal region) as well as in the whole-body 72 hours after therapy. In addition, the possible influence of other factors that may affect micronuclei (MN) frequency, such as age, gender, smoking habits, and histological type of tumour was analyzed. The study population consisted of 22 DTC patients and 20 healthy donors. Data on the distribution of 131-I were obtained from the whole-body scans. MN frequency and cytokinesis-block proliferation index (CBPI) were measured using cytokinesis-block micronucleus assay. 131-I therapy significantly increased the MN frequency (19.50 ± 6.90 vs. 27.10 ± 19.50 MN) and significantly decreased the CBPI (1.52 ± 0.20 vs. 1.38 ± 0.17) in patients' lymphocytes. There was a clear correlation between the increased MN frequency and 131-I accumulation in the thyroid region in patients without metastases. The MN values did not differ in relation to the factors that could affect MN, such as age, gender, smoking habits, and histological type of tumour. In conclusion, the MN frequency in PBLs of DTC patients without metastases depends on the accumulation of 131-I in the thyroid region and does not depend on the other factors examined.

Keywords: differentiated thyroid cancer; micronuclei; peripheral blood lymphocytes; radioactive iodine; whole-body scintigraphy
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Differentiated thyroid cancers (DTCs) derive from follicular cells of the thyroid and include papillary and follicular cancers (Boyce 1995). In patients with DTCs, the initial treatment includes total or near-total thyroidectomy (Schlumberger and Sherman 2012) and radioactive iodine (131-I) therapy. The purpose of 131-I therapy is to ablate remnant thyroid tissue and treat iodine-avid metastases (Pacini et al. 2005; Sawka et al. 2008). According to the American Thyroid Association (ATA) recommendations, after total thyroidectomy, 131-I administration is indicated in patients with moderate to high risk of recurrence based on age, tumour size, lymph node status, extrathyroidal extension, and the histological type of the thyroid tumour (Cooper et al. 2009; Schlumberger and Sherman 2012). However, the significant radiation doses delivered to extrathyroidal tissues are associated with acute and long-term risks and side effects, including second primary malignancies (Rubino et al. 2003; Brown et al. 2008; Sawka et al. 2009).

There are two general approaches in radioiodine therapy of DTC patients. The first involves administration of standard activities of I3I-I. The second approach involves administration of a tracer amount of 13I-I to determine the individual patient radioiodine pharmacokinetics and lesion uptake (Furhang et al. 1999). After ingestion, 131-I is absorbed from the gastrointestinal tract into the bloodstream and accumulated in thyroid tissue (Stabin 2003; Robbins and Schlumberger 2005). In the absence of excessive iodine, the uptake of 131-I in thyroid cells is mainly related to thyroid cell differentiation and the intensity of stimulation with thyroid-stimulating hormone (TSH) (Remy et al. 2008). The remainder is quickly excreted in faeces and urine or gradually eliminated from the body (Hänscheid et al. 2006; Sisson et al. 2011). The absorbed

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dose and dose rate to the blood could be calculated with several types of measurements: whole-body measurements with a survey meter, gamma camera obtained whole-body images, blood and urinary samples measurements (Furhang et al. 1999; Hänscheid et al. 2006, 2009; Remy et al. 2008). To assess the absorbed dose, Furhang et al. (1999) recommended one measurement at the uptake phase, an additional measurement during the typical peak time and a final measurement as late as possible (72-96 h), while Hänscheid et al. (2009) used a simple method with a single external measurement of the whole-body retention after the 131-I therapy. In patients with thyroid cancer who commonly had low 131-I uptake Hänscheid et al. (2006) recommended ROI analysis of gamma camera obtained whole-body images.

Iodine-131 administration and incorporation causes protracted and continuous internal whole-body irradiation (gamma radiation of 0.38 MeV photons, and beta radiation of 0.19 MeV electrons; half-life 8.04 days). One of the cytological consequences of chromosomal damage caused by radiation injury is the formation of micronuclei (MN). MN are chromosomal fragments or whole chromosomes not included in the nuclei during division but present in the cytoplasm of daughter cells as small additional nuclei (Fenech et al. 2011). Numerous published studies showed an increase of MN frequency in PBLs of DTC patients treated with 131-I (Livingston et al. 1993; Watanabe et al. 1998; Ballardin et al. 2002; Hooman et al. 2008). The published results showed discrepancies in the relationship between the administered 131-I activity and MN frequency. Several studies showed a dose-independent increase in MN frequency in patients with DTC treated with 131-I (Gutierrez et al. 1999; Monsieurs et al. 2000; Joseph et al. 2009), while Ballardin et al. (2002) showed a dose-dependent effect of 131-I on MN frequency. Because some of the applied iodine specifically bind and are retained in the residual thyroid tissue, while some iodine persist in other parts of the body for days after the therapy, giving very heterogeneous radiation exposure in patients treated with the same dose of 131-I (Hänscheid et al. 2006, 2009), the aim of our study was to examine whether the intensity of DNA damage measured by the cytokinesis-block micronucleus assay depends on the amount of 131-I that persists in the selected regions of interest (thyroid and abdominal region) as well as in the whole-body of DTC patients after therapy with two fixed activities (3.7 or 5.5 GBq) of 131-I. Besides, we wanted to investigate the possible influence of other factors that may affect MN, such as age, gender, smoking habits, and histological type of tumour.

Material and Methods

Study population

The study was approved by the Ethical Committee of the Clinical Centre Kragujevac. All patients and control subjects provided their written consent, according to the Declaration of Helsinki.

The study population included 22 well-differentiated thyroid cancer patients (15 females and 7 males), with the mean age of 51.7 ± 11.8 years (ranging from 38 to 76 years). Out of 22 DTC patients, 17 (77.3%) had papillary carcinoma, and 5 (22.7%) had the follicular variant of papillary carcinoma. Twelve patients had no clinical evidence of metastases, while 10 patients had metastases in the lymph nodes (9 patients) or bone (1 patient). Four to six weeks after surgical (total) thyroidectomy and 10 days after a low iodine diet, according to EANM guidelines (Luster et al. 2008), the patients were treated with fixed nominal activities of 3.7 GBq (100 mCi) (12 patients) or 5.5 GBq (150 mCi) (10 patients) of orally administered sodium iodide (131-I) at the Nuclear Medicine Department, Clinical Centre Kragujevac. At the time of 131-I administration, all 22 patients were hypothyroid after thyroid hormone withdrawal (TSH > 30 mIU/L). TSH values ranged from 31 to 359 mg/L (162.71 ± 98.78 mIU/L). The serum concentrations of thyroglobulin before the treatment ranged from 0.1 to 38 mg/L, with the mean value of 8.51 ± 10.66 mg/L. None of the patients had been exposed to potentially confounding factors, such as exposure to other ionising radiation (radiographic examination or scintigraphy) within 3 months before 131-I therapy. Fourteen of the 22 patients were smokers. The patients were released from the hospital at least 3 days after 131-I therapy or when the residual activity reached a value below 2 mR/h.

The control group consisted of 20 healthy subjects, 16 (80%) females and 4 (20%) males, with the mean age of 47.25 ± 13.25 years (range 30-70 years). They were colleagues and relatives who were willing to engage in the study and who had not been exposed to radioactive sources or other known genotoxic agents within at least 3 months. Eight of them were smokers.

Cytokinesis-block micronucleus assay

Heparinised blood was collected by venipuncture from 22 patients before and 7 days after 131-I treatment. The micronucleus assay was performed in PBLs using the cytokinesis-block micronucleus (CBMN) method described by Fenech and Morley (Fenech and Morley 1985). Whole heparinised blood (0.5 ml) was added into 5 ml of PBMax Karyotyping complete medium for lymphocyte culture (Invitrogen, California, USA). All cultures were carried out in duplicate and incubated at 37°C for 72 h.

Cytokinesis was blocked using 4 μg/ml of cytochalasin B (Sigma, St. Louis, MO, USA) 44 h after PHA stimulation. Cultures were harvested 28 h later. The cells were collected by centrifugation and re-suspended in a cold (4°C) hypotonic solution (0.56% KCl). Then, the cells were incubated in fresh Cornoy's fixative (acetic acid: methanol = 1 : 3) three times. The cell suspensions were dropped onto clean slides, air-dried, and stained with 2% Giemsa for 12 min (Alfapanon, Novi Sad, Serbia). The scoring was performed using a light microscope (Nikon E50i) at 400 × magnification and following the criteria for MN scoring only in binucleated (BN) cells described by Fenech (2000). The MN frequency was determined by analysing 1,000 BN cells per person.

A thousand viable cells from each patient were scored to determine the frequency of cells with 1, 2, 3, or 4 nuclei and to calculate the cytokinesis-block proliferation index (CBPI) using the formula CBPI = (M1 + [2 × M2] + 3 × [M3 + M4])/N, where M1 - M4 represent the number of cells with 1-4 nuclei, respectively, and N being the total number of viable cells scored (Fenech et al. 2003).

Whole-body scintigraphy

Iodine-131 whole-body scintigraphy was performed in all

patients 72 h after its application using a Siemens ECAM Dual Head scintillation camera (Siemens Medical Solutions USA) equipped with high-energy collimators, set to the 131-I peak at 364 keV, with an energy window width ± 10%, in the matrix of 256 × 1,024 pixels for 40 min (scan speed 6 cm/min). The scintillation camera measured radioactive uptake at any focus of the body as well as the whole-body retention of 131-I. The regions of interest included the thyroid region (salivary glands not included) and abdomen (bladder not included). These regions were outlined using the e.soft software package (Syngo MI Applications 2009A ver:8.2.26.8_VE32B16P30 IR26.5) in AP and PA position. The detected radioactivity is shown as the mean radioactivity, the mean total, and the mean radioactivity in two regions of interest (thyroid and abdominal), obtained as the average number of counts recorded in the AP and PA positions.

Statistical analysis

The results are shown as the mean ± standard deviation (S.D.). The Mann-Whitney *U*-test was used for the comparison of micronuclei frequency and CBPI between controls and patients as well as between the two groups of patients depending on their therapeutic dose of 131-I. Statistically significant differences between the mean baseline and induced MN frequencies were determined using the Wilcoxon test. Spearman's correlation coefficient was used to determine the correlations between micronuclei frequency and patient age, smoking habits, gender, and tumour stage. *P* values less than 0.05 were considered significant, and those less than 0.01 were considered highly significant.

Results

Table 1 shows the characteristics of the thyroid cancer patients and the administered doses of 131-I.

Table 2 presents the mean number of counts for the whole-body scintigrams as well the mean number of counts in two regions of interest (thyroid and abdomen) obtained using the gamma camera accompanied with the e.soft software package. The number of counts obtained on the analyzed scintigrams demonstrated a high degree of diversity among the patients who received the same dose of 131-I. In the group of patients who received 3.7 GBq, the mean total number of counts ranged from 762,500 to 7,681,643, whereas in the group treated with 5.5 GBq, the mean total number of counts ranged from the minimum value of 1,513,000 to the maximum value of 14,294,000. The ratio between the minimum and maximum mean total number of counts was 10.07 in patients treated with 3.7 GBq of 131-I and 9.47 in patients treated with 5.5 GBq of 131-I.

Fig. 1 shows four representative whole-body scintigrams of DTC patients registered 72 h after 131-I treatment. The image shows the accumulation of 131-I in the AP and PA positions in patient No. 1 (stage: T2N0M0, TSH = 282 mIU/L, Tg = 4.5 mg/L) and No. 11 (stage: T1N0M0, TSH = 359 mIU/L, Tg = 21.6 mg/L) treated with 3.7 GBq as well as in two other patients, No. 13 (stage: T1N1M0, TSH = 62 mIU/L, Tg = 2.1 mg/L) and No. 18 (stage: T2N0M1, TSH = 142 mIU/L, Tg = 15 mg/L), treated with 5.5 GBq of

Table 1. Characteristics of the thyroid cancer patients treated with 131-I.

Patient no.	Age (y)	Sex (F/M)	Stage (TNM)	Histology (P/F)	Tg (mg/L)	TSH (mIU/L)
1	43	M	T2N0M0	P	4.5	282
2	45	F	T1N1M0	P/F	0.5	65.7
3	42	F	T1N0M0	P	0.1	180
4	47	F	T1N0M0	P	1.0	148
5	40	M	T1N1M0	P	10.4	71
6	48	M	T2N0M0	P	0.1	168
7	60	F	T1N0M0	P	0.4	85
8	56	M	T3N1M1	P	2.3	306
9	61	F	T1N1M0	P/F	16.3	138
10	76	F	T2N0M0	P	22	130
11	44	F	T1N0M0	P	21.6	359
12	63	F	T1N0M0	P/F	1.2	262
13	38	F	T1N1M0	P/F	2.1	62
14	66	M	T1N1M0	P	6.3	32.9
15	43	F	T1N0M0	P	16.4	170
16	43	F	T1N0M0	P	2.1	128
17	64	F	T1N0M0	P	0.1	31
18	42	F	T2N0M1	P	15	142
19	71	F	T2N0M0	P	0.1	155
20	41	M	T2N1M0	P/F	38	76.5
21	40	M	T1N1M0	P	1.4	364
22	64	F	T2N1M0	P	25.3	125.6

Table 2. Mean radioactivity in two regions of interests (thyroid and abdominal) and mean total radioactivity obtained from whole body scintigrams 72 hours after the radioiodine treatment (3.7 or 5.5 GBq of 131-I).

Patient no.	Dose (GBq)	ROI thyroid (counts)	ROI abdominal (counts)	Total (counts)
1	3.7	227,500	89,500	762,500
2	3.7	14,083	208,490	920,842
3	3.7	31,000	581,500	1,091,500
4	3.7	142,000	256,500	1,178,000
5	5.5	99,500	621,500	1,513,000
6	3.7	11,500	523,500	1,600,500
7	3.7	27,479	495,927	1,753,918
8	5.5	36,500	375,500	2,080,000
9	5.5	72,841	601,480	2,380,382
10	3.7	726,500	1,178,500	3,221,000
11	3.7	72,841	1,429,332	3,240,314
12	3.7	791,214	1,150,183	4,228,839
13	5.5	1,445,000	781,500	4,460,000
14	5.5	1,905,000	708,500	4,832,000
15	5.5	633,000	1,961,500	5,422,000
16	3.7	1,745,121	1,078,651	6,491,364
17	3.7	129,979	1,998,972	6,911,296
18	5.5	529,948	1,519,184	7,555,137
19	3.7	133,032	3,025,719	7,681,643
20	5.5	607,500	1,741,500	8,651,500
21	5.5	134,500	3,258,000	9,665,000
22	5.5	618,000	3,588,500	14,294,000

131-I.

The results of the MN and CBPI analyses in the PBLs of patients and healthy controls are shown in Tables 3-6.

The baseline MN frequency in patients ranged from 7 to 33/1,000 analyzed BN cells. After the therapy, the lowest micronucleus frequency was 11/1,000 BN, and the highest was 48/1,000 BN cells. The CBPI values ranged from 1.20 to 2.17 before and from 1.18 to 1.99 after the therapy. In the healthy controls, the baseline MN frequency varied from 0 to 10 MN/1,000 BN cells, and the CBPI values varied from 1.42 to 1.69 (Table 3).

The summarised results of the MN frequency and CBPI analyses in the DTC patients and control subjects are shown in Table 5. The mean baseline MN frequency in DTC patients was significantly higher in comparison to the controls (Mann-Whitney test, 19.50 ± 6.90 vs. 4.40 ± 2.45, $p < 0.001$) and was also higher 7 days after the application of 131-I compared to the baseline values of the patients (Wilcoxon test, 27.10 ± 9.80 vs. 19.50 ± 6.90, $p < 0.001$). In the group of patients treated with 5.5 GBq of 131-I, both the mean baseline and induced MN frequency were slightly higher than in the group treated with 3.7 GBq (before therapy 20.90 ± 8.20 vs. 18.40 ± 5.70 and after therapy 28.70 ± 11.43 vs. 25.75 ± 8.70), but without statistical significance (Mann-Whitney test, $p_{before} = 0.390$; $p_{after} = 0.446$).

The baseline CBPI values in the patients were significantly lower in comparison to the controls (Mann-Whitney test (1.52 ± 0.20 vs. 1.55 ± 0.06, respectively; $p = 0.036$). After the therapy, the mean CBPI values significantly decreased from 1.52 ± 0.20 to 1.38 ± 0.17 ($p < 0.001$). There was no significant difference in the mean CBPI values between the groups of patients treated with the two different doses of 131-I (Wilcoxon test, $p = 0.590$). Binary logistic regression analysis excluded a statistically significant effect of ordinate doses on the CBPI values ($p = 0.301$).

The MN values did not differ in relation to patient age ($p = 0.825$), gender ($p = 0.463$), the histological type of DTC ($p = 0.597$), or the presence of metastasis ($p = 0.356$). Smokers had higher baseline and induced MN values in comparison to non-smokers, but these results were not statistically significant (binary logistic regression test, $p_{baseline} = 0.301$; $p_{induced} = 0.548$) (Table 4).

Spearman correlation coefficients did not show significant correlations between 131-I therapy-induced MN frequency and age ($p = 0.697$), gender ($p = 0.66$) or the presence of metastases ($p = 0.395$). Correlation analysis between the number of counts obtained from whole-body scintigrams and therapy-induced MN frequencies showed a positive correlation between the number of counts accumulated in the region of the thyroid gland and the MN frequency after the therapy, which was statistically significant in the group of patients treated with 3.7 GBq (bivariate correlation test, $p = 0.016$, Spearman $r = 0.674$). The number

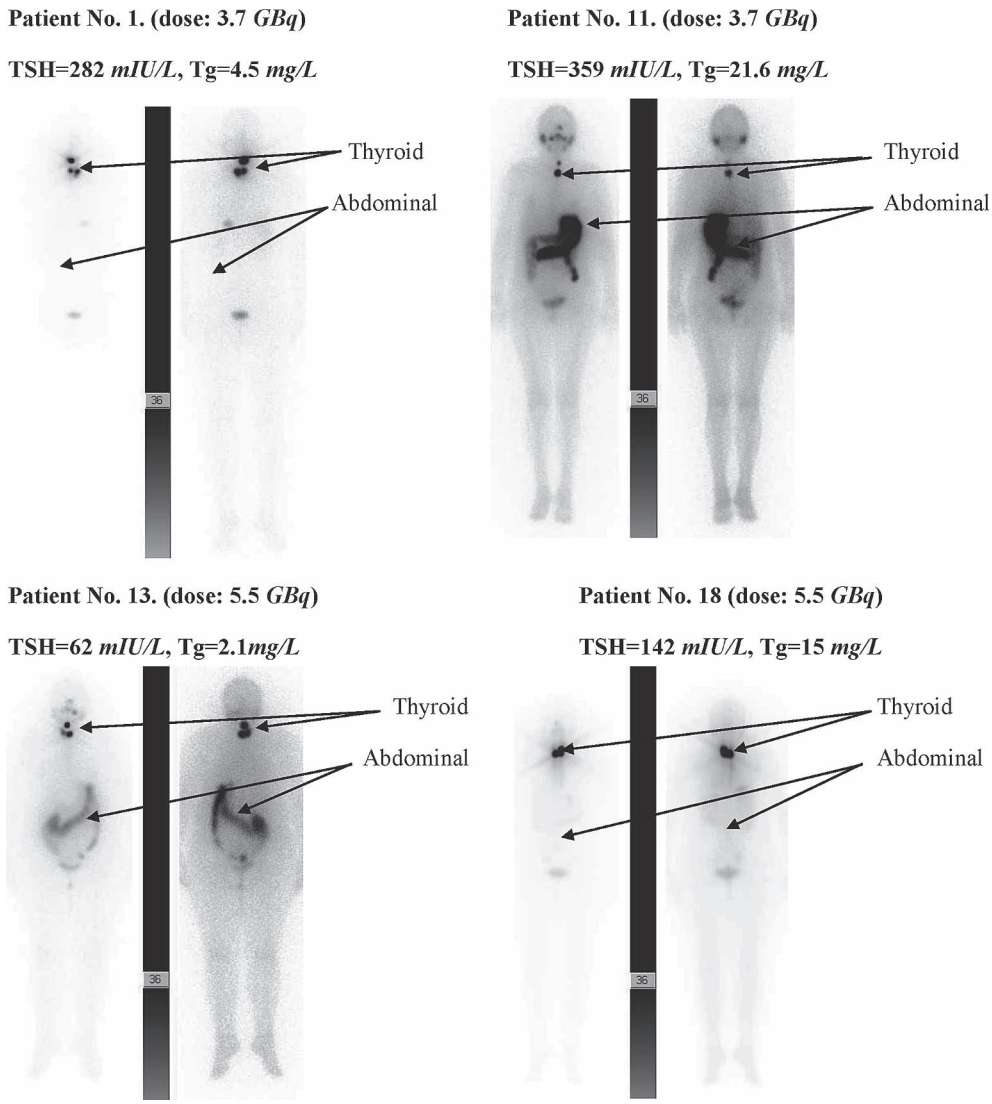

Fig. 1. Whole body scintigrams of four DTC patients.

of counts registered in the abdominal region of all patients correlated with the degree of hypothyroidism (bivariate correlation test, $p = 0.845$, Spearman $r = 0.045$). The mean total activity detected in the body of patients 72 h after radioiodine therapy did not have a significant effect on the MN frequency after the treatment (bivariate correlation test, $p = 0.252$, Spearman $r = 0.255$) or on the CBPI values in the PBLs (bivariate correlation test, $p = 0.693$, Spearman $r = -0.089$).

Table 6 shows an increase in the MN distribution in BN cells after the therapy compared to the baseline frequency of both patients and healthy controls. The frequency of BN cells with 1 MN after the therapy was 1.90% in comparison to 1.29% before the therapy and 0.38% in controls. There were fewer BN cells with 2 MN in all studied populations of BN cells (0.27% after therapy vs. 0.20 % before therapy and 0.03% in controls), while BN cells with 3 and 4 MN were found only in the patient samples (before and after the therapy).

Discussion

The principal result of the present study is the distribution of 131-I in DTC patients 3 days after the therapy with two fixed doses of radioiodine and its impact on the cytogenetic damage assessed by MN frequency. In addition, the other factors that may affect the MN frequency such as the dose of 131-I, histological type of the tumour, and presence of metastases, the age, gender, and smoking habits of the DTC patients were analyzed.

The analysis of selected regions of interest in gamma-camera images has already used (Siegel et al. 1999; Furhang et al. 1999). Postablation whole-body iodine scans are usually performed 3-7 days after the administration of 131-I (O'Neill et al. 2010). Furhang et al. (1999) recommended ROI analysis for determination of individual patient uptake and clearance of radioiodine with the final measurement as late as possible (72-96 h). In this study we analyzed whole-body images obtained 72 h after the 131-I

Table 3. Baseline and 131-I -induced micronuclei frequency and cytokinesis block proliferation index in peripheral blood lymphocytes of patients with differentiated thyroid cancer.

Patient no.	Age (years)	Sex (F/M)	Smoking (+/−)	Dose (GBq)	Before therapy		After therapy	
					MN/1,000 BN cells	CBPI	*MN/1,000 BN cells*	CBPI
1	43	M	+	3.7	22	1.77	35	1.51
2	45	F	−	3.7	16	1.49	21	1.41
3	42	F	−	3.7	14	1.47	18	1.33
4	47	F	+	3.7	18	1.36	24	1.29
5	40	M	+	5.5	16	1.55	21	1.35
6	48	M	+	3.7	7	2.17	11	1.99
7	60	F	+	3.7	15	1.2	32	1.18
8	56	M	−	5.5	22	1.5	29	1.44
9	61	F	−	5.5	29	1.44	37	1.17
10	76	F	+	3.7	20	1.35	23	1.3
11	44	F	−	3.7	17	1.5	22	1.43
12	63	F	+	3.7	17	1.51	23	1.27
13	38	F	+	5.5	12	1.49	16	1.37
14	66	M	−	5.5	15	1.33	19	1.32
15	43	F	+	5.5	26	1.53	32	1.42
16	43	F	+	3.7	28	1.62	41	1.59
17	64	F	+	3.7	20	1.35	22	1.31
18	42	F	−	5.5	33	1.74	48	1.31
19	71	F	−	3.7	27	1.7	37	1.38
20	41	M	+	5.5	29	1.37	44	1.28
21	40	M	+	5.5	19	1.48	25	1.35
22	64	F	+	5.5	8	1.54	16	1.36

Table 4. Micronuclei frequency and cytokinesis block proliferation index in peripheral blood lymphocytes of control subjects.

Control no.	Age (y)	Sex (F/M)	Smoking (+/−)	*MN/1,000 BN cells*	*CBPI*
1	38	F	+	5	1.61
2	64	F	+	5	1.51
3	44	F	+	6	1.51
4	61	F	−	5	1.55
5	30	F	−	3	1.57
6	30	M	−	0	1.69
7	52	F	−	5	1.52
8	79	F	−	10	1.51
9	36	F	−	2	1.51
10	31	M	−	3	1.42
11	56	F	−	7	1.50
12	59	F	−	6	1.49
13	50	F	+	6	1.60
14	53	F	+	8	1.58
15	49	F	+	3	1.56
16	40	M	+	2	1.53
17	30	F	−	2	1.52
18	51	F	−	4	1.55
19	54	M	−	1	1.62
20	38	F	+	5	1.60

Table 5. Sumarized results of micronuclei frequency and cytokinesis block proliferation index in analyzed patients (n = 22) and healthy controls (n = 20).

Analysis	Patients					Controls		
	n	MN frequency		CBPI		n	MN *frequency*	CBPI
		before *131-I*	after *131-I*	before *131-I*	after *131-I*		mean ± S.D.	
		mean ± S.D.		mean ± S.D.				
Mean age *total* Age range	22	51.70 ± 11.80 38-76				20	47.25 ± 13.5 30-70	
total		19.50 ± 6.90[a]	27.10 ± 19.50[b]	1.52 ± 0.20[c]	1.38 ± 0.17[d]		4.40 ± 2.45	1.55 ± 0.06
Gender								
Males	7	18.00 ± 7.31	25.85 ± 11.50	1.59 ± 0.30	1.46 ± 0.24	4	1.50 ± 1.29	1.56 ± 0.11
Females	15	20.26 ± 6.85	27.80 ± 9.42	1.48 ± 0.14	1.34 ± 0.11	16	5.12 ± 2.12	1.54 ± 0.04
Smoking								
Yes	14	21.30 ± 6.80	28.70 ± 10.2	1.52 ± 0.23	1.35 ± 0.08	8	5.00 ± 1.85	1.56 ± 0.04
No	8	18.40 ± 6.90	26.20 ± 9.90	1.52 ± 0.13	1.39 ± 0.19	12	3.92 ± 2.74	1.54 ± 0.07
Therapy doses (GBq)								
3.7	12	18.40 ± 5.70	25.75 ± 8.70	1.54 ± 0.27	1.41 ± 0.23	/		
5.5	10	20.90 ± 8.20	28.70 ± 11.43	1.49 ± 0.14	1.33 ± 0.08	/		
Metastasis								
Yes	10	21.00 ± 8.17	29.10 ± 11.2	1.51 ± 0.09	1.35 ± 0.08	/		
No	12	18.33 ± 5.73	25.58 ± 8.80	1.52 ± 0.25	1.41 ± 0.21	/		
Tumor stage								
T1N0M0/N1M0	14	18.71 ± 5.28	25.20 ± 7.43	1.41 ± 0.11	1.34 ± 0.11	/		
T2N0M0/N1M0/N0M1	7	20.85 ± 10.09	30.57 ± 14.1	1.66 ± 0.28	1.45 ± 0.25	/		
T3N1M1	1	22	29	1.5	1.44	/		

a) $p < 0.001$ vs. control., b) $p < 0.001$ vs. before *131-I* , c) $p = 0.036$ vs. control., d) $p < 0.001$ vs. before *131-I.*

Table 6. Distribution of micronuclei in thyroid cancer patients (before and after therapy) and control subjects.

	Distribution of MN (%)			
	1 MN	*2 MN*	*3 MN*	*4 MN*
Thyroid cancer patients before radioiodine therapy	283 (1.29)	44 (0.2)	17 (0.07)	2 (0.01)
Thyroid cancer patients after radioiodine therapy	416 (1.9)	61 (0.27)	16 (0.07)	3 (0.01)
Control subjects	77 (0.38)	6 (0.03)		

BN, binucleated; MN, micronuclei.

administration. In the group of patients receiving the same dose of 131-I (3.7 or 5.5 GBq), a higher activity detected in the selected ROIs and whole bodies indicates the slower clearance of 131-I.

The accumulation of 131-I in thyroid region of our DTC patients after radioiodine treatment indicates that these patients have a significant residual iodine-avid tissue despite surgical total thyroidectomy. Great individual differences in the number of counts registered in the thyroid regions of patients who were treated with the same dose of 131-I (3.7 or 5.5 GBq) could possibly be explained by differences in the amount of residual thyroid tissue after the surgery, as well as by differences in ability of cancer cells to uptake iodine and/or by different levels of TSH after thyroid hormone withdrawal. In the conditions of low iodine diet, the uptake of iodine in thyroid cells is mainly related to thyroid cell differentiation and the intensity of stimulation with TSH. However, most of the patients had a very large amount of remnant radioactivity in the gastrointestinal tract (stomach and colon), most likely because the patients were severely hypothyroid (TSH ranged from 31 to 359, 162.71 ± 98.78 mIU/L) prior to radioiodine therapy, which could be associated with the delayed transit of contents through the intestinal tract (Kita et al. 2004). In addition, during hypothyroidism after thyroid hormone withdrawal, renal clearance of radioiodine decreases, potentially prolonging its retention in the body (Remy et al. 2008). Hence it is not surprising that great individual differences occur in the number of counts in the region of the gastrointestinal tract, as well as in the total number of counts between the

groups of patients treated with the same dose of 131-I. Large variations in the total radioactivity measured within the two groups of our patients (treated with 3.7 and 5.5 GBq, respectively) 72 h after the application of 131-I and considerable overlap between the values measured in patients who were treated with the two different doses of 131-I could explain the lack of influence of dose on MN frequency. The dose-independent increases of MN frequency in PBLs of DTC patients treated with 131-I were reported by Gutierrez et al. (1999), Monsieurs et al. (2000), and Joseph et al. (2009), while Ballardin et al. (2002) showed a dose-dependent effect of 131-I on MN frequency.

Previous studies show an increased DNA damage in the PBLs of DTC patients treated with 131-I (Livingston et al. 1993; Watanabe et al. 1998; Ballardin et al. 2002; Hooman et al. 2008). However, our study is the first to examine whether the frequency of MN in the PBLs of radioiodine-treated DTC patients depends on the retention of radioiodine in the selected parts, as well as in whole patient's body after the therapy. The blood is irradiated by β-particles emitted from circulating 131-I and from penetrating γ-radiation, originating from the activity dispersed throughout the body (Lassmann et al. 2010). In PBLs of 131-I-treated patients chromosomal aberrations (Baugnet-Mahieu et al. 1994; Gundy et al. 1996; Monteiro et al. 2000; Puerto et al. 2000), the MN frequency (Livingston et al. 1993; Watanabe et al. 1998; Ballardin et al. 2002; Lee et al. 2002; Hooman et al. 2008) and the CBPI (Gutirrez et al. 1995; Ramirez et al. 1997) were analyzed.

In our DTC patients, the baseline MN frequency was higher in comparison to the MN frequency in the healthy controls. This result is in agreement with the previously published conclusions that the baseline MN frequency is higher in thyroid cancer patients (Iarmarcovai et al. 2008) and in patients with other types of tumours (Baciuchka-Palmaro et al. 2002; Bonassi et al. 2007; Murgia et al. 2008; Milošević-Djordjević et al. 2010) than in healthy persons. Similarly, the increase in the mean MN frequency in PBLs of patients after 131-I therapy is consistent with the previous publications (Ballardin et al. 2002; Popova et al. 2005). The presented data show a significant increase in BN cells with 1 MN in the PBLs of patients compared to controls. In addition, the data show that the BN cells with 3 and 4 MN were observed only in patient samples (before and after the therapy). At the same time, the baseline CBPI values were significantly lower in the patients than in the controls, and these values decrease further after the application of 131-I. This result is in agreement with the results reported by Ramirez et al. (1997) for thyroid cancer patients and Jianlin et al. (2004) for other types of tumours.

In the group of DTC patients treated with 3.7 GBq of 131-I, a correlation between the MN frequency and the number of counts registered in the thyroid region was observed, but a correlation between the MN frequency and total body radioactivity was not found. The total radioactivity clearly had less impact on the MN frequency in comparison to the radioactivity accumulated in the thyroid region. These findings could possibly be explained by the greater intensity of irradiation of PBLs passing through the tumour tissue, which is accompanied by greater cytogenetic damage that may be sufficient for the formation of increasing numbers of MN in these cells.

Our results show that age, gender, smoking habits, and the histological type of the tumour did not affect baseline or 131-I-induced MN in the analyzed patients. These results are in agreement with the results reported by Milosevic-Djordjevic et al. (2010) who investigated the relationships among age, gender, smoking habits, cancer site, and MN frequency in the lymphocytes of patients with different types of cancers.

Our results indicate that 131-I therapy induces DNA damage in peripheral blood lymphocytes of DTC patients regardless of age, gender, smoking habits, and the histological type of tumour. In patients without metastases, MN frequency after 131-I therapy correlates with the accumulation of 131-I in the thyroid tissue. Abdominal as well whole-body retention of 131-I does not significantly affect the MN frequency or CBPI values in PBLs of DTC patients.

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Conflict of Interest

The authors declare they have no conflicts of interest.

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