Journal of BUON **14:** 85-91, 2009

ORIGINAL ARTICLE

Granulocytes' enzymes as biomarkers of radiotoxicity in individuals occupationally exposed to low-level radiation

S. Milacic

Faculty of Medicine, University of Belgrade, Institute of Occupational Medicine and Radiological Protection, Belgrade, Serbia

Summary

Purpose: *To investigate the possibility of using the granulocytes' enzymes alkaline phosphatase (L-ALP) and myeloperoxidase (MPO) as biomarkers to study and analyse contamination of nuclear medicine personnel working with radionuclides (RN) when radiotoxic effects are very low, before occupational radiation illness or benign haematological disorders and malignant diseases have occurred. Also, to investigate the relationship between chromosomal aberrations in lymphocytes and the activity of L-ALP and MPO in neutrophil granulocytes (NphG).*

Materials and methods: *The absorbed external doses of ionizing radiation (IR) were measured by thermoluminescent personal dosimeters (TLD) for the duration of occupational exposure (DOE). Urine radioactivity was measured by γ-spectrometry. Venous blood was used for leukocyte count and search for chromosomal aberrations by conventional cytogenetic techniques. Blood smears were stained for L-ALP and MPO using a modified Kaplow's method and the classical method with benzidine dihydrochloride, respectively. The occupationally exposed group (E) consisted of 74 workers exposed to short-life radioactive isotopes I131 (β and mostly γ emission) and mTc99 (γ emission). The control group (C) consisted of 52 subjects living in the same region, working in the same institution, occupationally not exposed to RN. A patients' group (P; n=31) took I131 or mTc99 for diagnostic purposes.*

Results: *Although the measured values did not exceed the yearly quota for professionally exposed individuals, characteristic chromosomal aberrations in peripheral blood lymphocytes (dicentrics, fragments, rings) were identified. L-ALP and MPO activity was inhibited in the NphG in occupationally exposed workers, especially in persons with chromosomal aberrations, working for a long time in ionizing radiations zones ($p < 0.01$).*

Conclusion: *Decreased activity of L-ALP and MPO can reveal effects of long-lasting exposure to low-dose IR. A significant relationship between chromosomal aberrations in lymphocytes and activity of the enzymes in granulocytes was found.*

Key words: chromosomal aberrations, granulocytes' enzymes, leukocyte alkaline phosphatase, low level ionizing radiation, myeloperoxidase

Introduction

Radiotoxicity depends not only on the toxic properties but also on the dose of IR of RNs administered (in patients) or penetrating into the body (in occupationally exposed persons), including also the type of the radioactivity emission.

Long-term exposure to IR in nuclear medicine workers would cause numerous cells disorders [1-3]. In case that a RN reaches the organism it causes internal irradiation and lesions to sensitive tissues.

Sensitive markers of pathological damage of cells by IR are chromosomal aberrations in lymphocytes, used as biodosimeters or as biomarkers of low level IR [2,3].

White blood cell enzymes such as L-ALP and MPO are also sensitive indicators for radiotoxic effects [4]. L-ALP and MPO are located in the cytoplasmic granules of NphG.

MPO-positive granules are azurophilic or primary, and L-ALP-positive granules are secondary granules in NphG. Deficiencies of primary and secondary granules indicate disorder of maturation of precursor cells in the bone marrow and they consequently have been used in the identification of leukaemia cells [5,6].

Correspondence to: Snezana Milacic, MD, PhD. Faculty of Medicine, University of Belgrade, Institute of Occupational Medicine and Radiological Protection, Deligradska 29, 11000 Belgrade, Serbia. Tel: + 381 11 3674 515, Fax: + 381 11 643 675, E-mail: m.masha@beotel.net

Received 12-05-2008; Accepted 10-07-2008

L-ALP is a well known marker of chronic myeloid leukaemia (CML), but recent investigation suggests its usefulness for the diagnosis of several diseases [6].

Increased concentrations of L-ALP indicate inflammation, while abnormally low concentrations usually are related to genetic conditions or haematological illnesses (e.g. chronic myelogenous leukemia/CML, and myelodysplastic syndrome) or inhibition of white blood cells maturation because of toxins [4-9].

Deficiency of MPO, synthesized during the promyelocytic stage of haematopoiesis, indicates acute promyelocytic leukaemia (APL). CML is a leukaemia with deletion and translocation from chromosome 9 to 22 (t: 9; 22, Philadelphia chromosome), and APL is a neoplasm with a unique chromosomal translocation from 15 to 17 (t: 15; 17) [10-12].

MPO is part of the host defense system of granulocytes, responsible for microbiocidal activity against a wide range of harmful microorganisms. MPO is located in the cell nucleus as well as in the cytoplasm. Intranuclear MPO may help protect DNA against damage resulting from oxygen radicals produced during myeloid cell maturation and function. Decreased MPO may be associated with increased risk for many cancers, including lung, breast, bladder, and stomach [13]. MPO is elevated in acute coronary syndromes [14], inflammation, chest pain due to coronary disease, recurrent infections, taking antibiotics, and radioactive contamination [7,13,14]. Also MPO has been implicated in Alzheimer's disease [15].

IR can lower the leukocyte count, depending on its dose. Small doses do not necessarily result in decreased leukocyte count.

However, small doses of IR may result in DNA breaks and chromosomal lesions linked with inhibition of enzymes, including L-ALP and MPO [4,16-19]. In those studies deletion, translocation, dicentric or ringed chromosomes, and gene rearrangements in cells of bone marrow were connected with absence of activity of L-ALP and MPO due to inactivation of genes responsible for their synthesis. Also, enzymes already synthesized in mature blood cells can be neutralized due to low doses of IR.

In the present study the relationship between chromosomal aberrations in lymphocytes (dicentric) and activity of L-ALP and MPO in NphG were investigated with an aim to clarify whether they could help assess health risks connected with chronic low-dose IR.

Materials and methods

Group E (study group) consisted of 74 workers exposed to short-life radioactive isotopes I^{131} (β and mostly γ emission) and mTc^{99} (γ emission). This population worked in nuclear medicine departments, had no inflammations or exposure to chemical toxins and drugs. Their DOE varied, so group E was subdivided in group E1 (1-5 years), E2 (6-15 years) and E3 (16-30 years). The average age was 41.3 years (range 25-60), and 81.08% were women.

The control group (C) consisted of 52 healthy subjects living in the same region, working in the same institution, occupationally not exposed to RN. Their average age was 34 years (range 25-60), and 75% were women.

The patients' group (P; n=31) was scanned with I^{131} or mTc^{99} for diagnostic purposes. Their scans were normal. They received 0.19 mSv dose of radiation. All of them were investigated immediately before and a few hours after the application of RN to compare enzymes' activity.

Individuals with hormonal and metabolic disorders as well as those taking medications or other chemical substances and smokers were excluded from the study.

Samples from 24h urine collection from nuclear workers (group E) and control subjects (group C) were measured by γ-spectrometry. By implementing this method natural and artificial RN were identified, as well as their origin, from environment and/or working places. Measurement of urine γ-radioactivity was used with the aim to set up the quantity of internal radioactivity [8,20].

The absorbed external doses of IR were measured by personal TLD for the DOE. TLD measurements were expressed as mean annual equivalent doses for the period of exposure in mSv per year [4,16].

Characteristic chromosomal aberrations (dicentric) indicate cumulative effects from both internal and external radiation doses and were studied in 200 peripheral blood lymphocytes in metaphase. Modified Moorhead's method [4,7,17] and conventional cytogenetic techniques were used for the preparation of lymphocytes. The cells in metaphase were microscopically examined in stained smears (5% May-Grunwald-Giemsa) under immersion microscope (magnification 100×16) [2,3].

Heparinized venous blood was used for the leukocyte count using an automatic counter. Blood smears stained with 5% May-Grunwald-Giemsa were studied through immersion microscope to determine the percent and absolute number of NphG, eosinophil granulocytes (EoG), basophil granulocytes (BsG), rod granulocytes (RG), monocytes (Mo) and lymphocytes (Ly).

Blood smears were also stained for L-ALP using a modified Kaplow's method [4,8,24].

A colorless cytoplasm was marked 0, a mildly stained 1, a clearly stained 2, that with numerous gran-

ules 3, and an intensely stained 4 (Figure 1). Every hundred of counted cells was multiplied by the stain intensity grade. The sum of these products made the L-APL score, i.e. the level of the enzyme activity.

Blood smears were also stained for MPO using the classical method with benzidine dihydrochloride, and the interpretation was modified as follows [4,21]: according to the stain intensity i.e. brown granules in the cytoplasm, each hundred of granulocytes was graded from 0 to 3. The sum of the products of the stain grade and the cell count made the score of the MPO activity (Figure 2).

The scores of both enzymes activity was expressed in international units (IU).

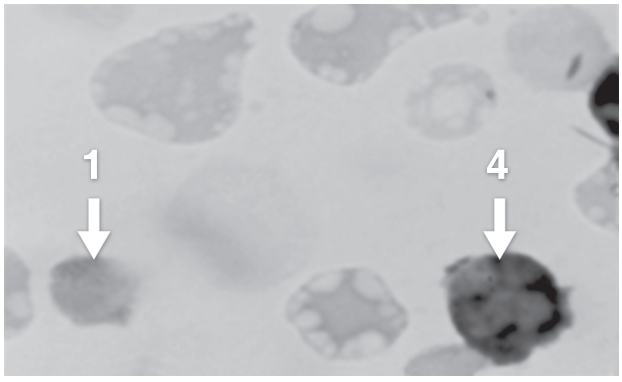

Figure 1. Activity of leukocytes' alkaline phosphatase marked 1 (left arrow) and 4 (right arrow) (modified Kaplow's method ×1,600).

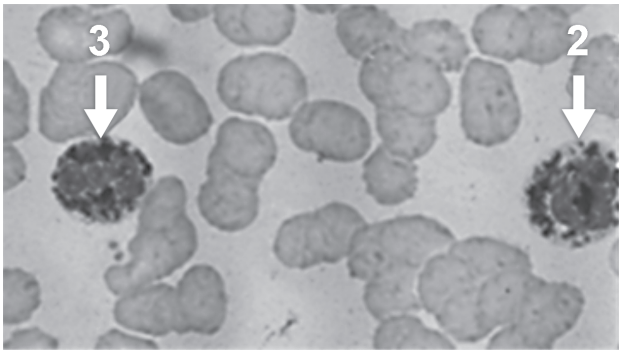

Figure 2. Activity of leukocytes's myeloperoxidase marked 3 (left arrow) and 2 (right arrow) (benzidine dihydrochloride ×1,600).

Statistical analysis

For statistical analysis Student's t-test and Student's pair t-test for processing the laboratory results and dosimeters data were used. For sum rank comparison, the Wilcoxon non parametric test and Kruskal-Wallis test were used for processing the enzyme activity score; Mann-Whitney's test of the rank sums was used to compare differences between two groups of nonparametric data per one characteristic (score-IU). Pearson's x^2 test, in the form of contingency tables, was used for the analysis of two attributive characteristics (to analyze chromosome aberrations). For identification of correlation, single linear correlation and multiple regression analysis were used.

Results

The average γ-radioactivity in the urine of the exposed workers group E was 11.80 Bq/l (11.22 Bq/l from working place: I^{131}, $_mTc^{99}$; and 0.58 Bq/l from environment: Cs^{137}) (Table 1). In the control group, however, γ-radioactivity in the urine was 0.66 Bq/l (from environment: Cs^{137}), and the average total dose was 0.66 Bq/l ($p > 0.05$). The exposed workers were contaminated taking into consideration that their urine radioactivity had been over 10 Bq/l on average, although the mean value was below the annual intake limit (AIL) for the occupationally exposed subjects (200-600 Bq/l urine depending on the RN type).

No significant differences in the total lymphocytes and monocytes count were noted, while NphG counts were lowest and EoG were higher in the exposed workers ($p < 0.01$; Table 2).

The time of exposure in the workers with chro-

Table 1. Urine γ-radioactivity

Group	*No. of persons*	*RN^1 (Bq/l)*		*RN^2 (Bq/l)*	
		Range	*Average*	*Range*	*Average*
Exposed	74	1.20-28.70	11.22	0.29-0.80	0.58
Control	52	0-0.01	0.001	0.10-0.90	0.66
p-value		p <0.0001		p >0.05	

[1]radionuclide from working place, [2]radionuclide from environment

Table 2. White blood cells in medical nuclear workers and control subjects

Group	*No. of persons*	*Le*	*NphG*	*RG*	*Ly*	*EoG*	*BsG*	*Mo*
		Mean ×10^9/l ± SD						
Exposed	74	5.8±1.5	3.5±1.1	0.047±0.01	2.00±0.59	0.20±0.15	0.013±0.07	0.18±0.15
Control	52	6.5±1.2	4.1±1.0	0.009±0.01	2.11±0.47	0.08±0.04	0.001±0.01	0.19±0.13
Student's t-test		-2.32	-2.96	0.96	-1.15	5.14	1.83	-0.33
p-value		0.022	0.003	0.33	0.24	0.0001	0.06	0.73

Le: leukocytes, NphG: neutrophil granulocytes, RG: rod granulocytes, Ly: lymphocytes, EoG: eosinophil granulocytes, BsG: basophil granulocytes, Mo: monocytes, SD: standard deviation

mosomal aberrations was longer ($p < 0.05$), but doses (measured by TLD) were not significantly different (Table 3).

No differences in the number of white blood cells in medical nuclear workers according to the frequency of chromosomal aberrations were identified ($p > 0.05$; Table 4).

No differences before and after administration of RN were seen in the different leukocyte forms (Student's pair t-test, $p > 0.05$; Table 5).

Exposed workers were compared taking into consideration the exposure intervals (Table 6). Group E1 had the lowest average equivalent dose (0.83 mSv), significantly lower than groups E2 and E3. Group E2 was different from group E1, except for higher equivalent dose (1.72 mSv) as well as for granulocytes number (NphG were lowest; EoG were higher), and both of them were not different from group E3.

The activity of the studied enzymes L-ALP and MPO was lower in the exposed workers compared with the control group ($p < 0.01$). L-ALP and MPO were decreased in the exposed workers with chromosomal aberrations ($p < 0.001$; Table 7).

Table 3. Time of exposure and equivalent dose in medical nuclear workers with and without chromosomal abberations (c.a.)

Group of medical nuclear workers	*No. of persons*	*Years* Mean±SD	*mSv*
Exposed with c.a.	31	12.83±911	1.38±0.65
Exposed without c.a.	43	7.72±7.71	1.10±0.73
Student's t-test		2.44	1.70
p-value		0.017	0.09

No significant differences were detected concerning the activity of the enzymes before and after diagnostic RN administration in the patients' group. Those patients showed no inhibition of the enzymes in NphG compared with the control group.

The activity of L-ALP and MPO in the exposed workers varied with DOE (Figures 1,2). Correlation of L-ALP and MPO activity with DOE was significant (Table 8; $p < 0.01$), but was not linear. Regression L-ALP was $r = -0.31$ ($p=0.007$), and regression MPO was $r = -0.35$ ($p=0.002$).

Figure 3 shows groups with 1-5 (E1), 6-15 (E2)

Table 4. Number of white blood cells in medical nuclear workers according to chromosomal aberrations (c.a.)

Group of medical nuclear workers	*No. of persons*	*Le*	*NphG*	*RG*	*Ly* Mean $\times 10^9/l \pm SD$	*EoG*	*BsG*	*Mo*
Exposed with c.a.	31	5.5±1.5	3.2±1.1	0.0015	1.96±0.53	0.20±0.12	0.006	0.13
Exposed without c.a.	43	6.1±1.4	3.6±1.0	0.071	2.02±0.63	0.20±0.17	0.018	0.22
Student's t-test		1.78	1.85	0.67	0.47	0.12	1.12	1.92
p-value		0.07	0.06	0.50	0.63	0.90	0.26	0.059

For abbreviations see footnote of Table 2

Table 5. Number of white blood cells in patients during diagnostic examination

Group	*No. of patients*	*Le*	*NphG*	*RG*	*Ly* Mean $\times 10^9/l \pm SD$	*EoG*	*BsG*	*Mo*
Before RN	31	6.12±1.08	4.21±0.92	0.01±0.00	2.02±0.47	0.09±0.06	0±0	0.19±0.12
After RN	31	6.61±1.00	4.19±0.80	0±0	2.16±0.42	0.07±0.03	0±0	0.17±0.11
Student's pair t-test		0.68	0.17	1.00	1.34	1.51		0.74
p-value		0.50	0.86	0.32	0.19	0.14		0.46

RN: radionuclide for diagnostic examination. For other abbreviations see footnote of Table 2

Table 6. White blood cells and equivalent dose in medical nuclear workers during the occupational exposure

Group	*No. of persons*	*DOE (years)*	*TLD mSv±SD*	*Le* $10^9/l \pm SD$	*NphG 109/l±SD*	*RG·*$10^9/l$ *Mean*	*Ly* $10^9/l \pm SD$	*EoG·* $10^9/l \pm SD$	*BsG·*$10^9/l$ *Mean*	*Mo·*$10^9/l$ *Mean*
E1	35	1-5	0.83±0.38	6.20±1.53	3.8±1.0	0.09	2.0±0.6	0.14±0.1	0.014	0.23
E2	19	6-15	1.72±0.65	5.38±1.46	3.0±1.0	0.02	1.9±0.5	0.26±0.1	0.013	0.14
E3	20	16-30	1.44±0.83	5.81±1.49	3.4±1.1	0.01	2.0±0.5	0.25±0.1	0.011	0.15
E1-E2: t-test; p			0.0001	0.063	0.013	0.56	0.75	0.010	0.91	0.10
E2-E3: t-test; p			0.25	0.37	0.30	0.24	0.75	0.93	0.86	0.93
E1-E3: t-test; p			0.0006	0.36	0.19	0.47	0.99	0.004	0.82	0.16

DOE: duration of occupational exposure, TLD: thermoluminescent dosimeters. For other abbreviations see footnote of Table 2

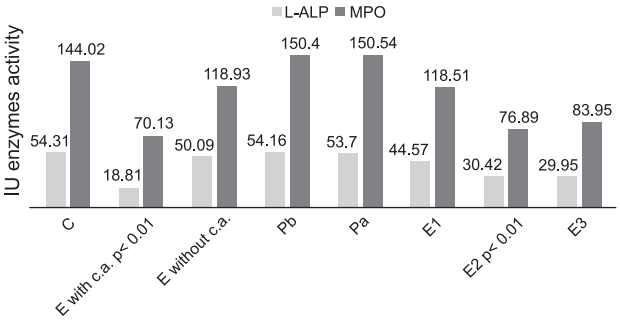

Figure 3. Leukocyte alkaline phosphatase (L-ALP) and myeloperoxidase (MPO). C: control group, E with c.a.: Exposed group with chromosomal aberrations, E without c.a.: Exposed group without chromosomal aberrations, Pb: Patients before radionuclide administration, Pa: Patients after radionuclide administration, E1: Exposed group with 1-5 years of DOE, E2: Exposed group with 6-15 years of DOE, E3: Exposed group with 16-30 years of DOE. Numbers represent enzymes activity in International Units.

and 16-30 (E3) years of DOE as well as exposed workers with and without aberrations, compared with the patient and control groups. The smaller activity of the investigated enzymes was identified in the group of exposed workers with chromosomal aberrations, as well as in those with 6-15 years of DOE (E2). Related to the enzymes activity (IU), group E2 differed significantly ($p < 0.01$) from group E1, but not from group E3. Workers without chromosomal aberrations were not different from the control and patient group ($p > 0.05$).

Discussion

It is well known that the biological effects of IR are chromosomal aberrations in lymphocytes, due to a combination of DNA damage and reduced DNA repair capacity [25]. However, exposure to low dose radiation also damages granulocytes and causes inhibition of L-ALP and MPO activity [3,4,7,8].

Since chromosomal aberrations act as biological indicators of the absorbed dose [22,23], we compared the exposed workers with positive findings with those without.

Despite the mean values of the leukocyte count and the other white blood cell components did not differ significantly in the subjects with and without chromosomal aberrations, the activity of the studied enzymes was significantly lower in workers with the chromosomal aberrations [4,7].

No changes were identified in patients who received one small diagnostic dose of RN. Changes were identified only in individuals working on diagnostic applications of the same RN after 6 and more years. The cumulative effects of small doses on the studied enzymes were different from the effects from one small dose.

Granulocytes' enzymes L-ALP and MPO significantly decreased in nuclear workers, especially in exposed workers with chromosomal aberrations, after long period of time (minimum 6 years of DOE).

Although granulocytes' counts varied in the exposed group (NphG were lowest; EoG were higher)

Table 7. Score of L-ALP and MPO in exposed workers, with or without chromosomal aberrations compared with both control group and patients

Group	*No. of persons*	*L-ALP score (IU)*			*MPO score (IU)*		
		Mean rank	*Z*	*p-value*	*Mean rank*	*Z*	*p-value*
Exposed	74	36.99	4.15	0.0001	98.49	4.88	0.0001
Controls	52	54.31			144.02		
Exposed with c.a.	31	18.81	5.55	0.0001	70.13	3.79	0.0001
Exposed without c.a.	43	50.09			118.93		
Patients before RN	31	54.16	0.45	0.65	150.54	0.09	0.92
Patients after RN	31	53.70			150.41		

c.a.: chromosomal aberrations, RN: radionuclide, IU: international units

Table 8. Correlations of L-ALP and MPO activity and number of white blood cells with time of exposure to ionizing radiation

Correlations to DOE	*N*	*L-ALP (IU)*	*MPO (IU)*	*NphG $\times 10^9$/l*	*EoG $\times 10^9$/l*	*Ly $\times 10^9$/l*
		Correlation coefficient (p-value)		*Correlation coefficient (p-value)*		
Exposed / DOE	74	-0.3093 (0.007)	-0.3466 (0.002)	-0.1679 (0.152)	0.2740 (0.018)	-0.0093 (0.937)
Exposed with c.a./DOE	43	-0.1265 (0.497)	-0.2821 (0.124)	-0.1448 (0.436)	0.5106 (0.003)	-0.0941 (0.615)

DOE: duration of occupational exposure, c.a: chromosomal aberrations. For other abbreviations see footnote of Table 2

compared with the controls, changes in the number of white blood cells did not correlate with exposure to low dose level of IR. We hypothesize that this might be a result of bone marrow stem cells' repair machinery.

The effects of RN were observed on sensitive molecules (L-ALP and MPO). Sensitive molecules (DNA, L-ALP and MPO) measured biological effects all the time during the exposure, as a result of both internal and external delivered doses. Chromosomal aberrations and granulocytes' enzymes show the cumulative effects of small doses received over a long time of exposure, indicate the relative radiation risk of carcinogenesis [23,26,27] and could be used for biological risk assessments. These changes of lymphocytes and granulocytes can negatively influence repair mechanisms, regeneration and redistribution of cells, contributing to the appearance of radiation lesions of tissues and initiation of malignant diseases [28,29].

Significant relationship between chromosomal aberrations and L-ALP and MPO deficiency was proven. No differences between the control group and the exposed group without aberrations related to the studied enzymes was observed.

Conclusion

The results of this study indicate that prolonged occupational exposure to low level radiation in nuclear medical laboratories can cause L-ALP and MPO inhibition. Owing to this effect cytochemical analysis of L-ALP and MPO in NphG in peripheral blood smears may be used for monitoring IR and health risk assessments.

The method is simple, quick and cheap, and is recommended to assess IR risk, together with other indicators, such as chromosomal aberrations.

References

1. Milacic S. Analysis of workers in nuclear medicine. Periodicum Biologorum 1989; 91: 417-418.
2. Günter S, Oestreicher U, Romm H. Biological Dosimetry In: Günter O (Ed): Chromosomal Alterations. Springer, Berlin, Heidelberg, 2007, pp 341-350.
3. Milacic S. The frequency of chromosomal lesions and damaged lymphocytes of workers occupationally exposed to x-rays. Health Phys 2005; 88: 334-339.
4. Milacic S. Cytological and cytochemical changes of leukocytes due to the internal radioactive contamination of the human body. (Dissertation) University of Belgrade, Medical Faculty, 1992.
5. Nauseef WM, Olsson I, Arnljots K. Biosynthesis and processing of myeloperoxidase-a marker for myeloid cell differentiation. Eur J Haematol 1988; 40: 97-110.
6. Pradella M, Barbasetti di Prun P, Nemetz L et al. A quantitative method to measure alkaline phosphatase activity in individual leukocytes by image analysis. Acta Histochem 1995; 97: 189-194.
7. Milacic S. Changes in leukocytes caused by tritium contamination. Health Phys 2004; 86: 457-459.
8. Milacic S, Petrovic D, Jovicic D, Kovacevic R, Simic J. Examination of health status of population from uranium contaminated regions. Environ Res 2004; 95: 2-10.
9. Sarah D, Caruthers MD. Focus on diagnosis: the alkaline phosphatase level: nuances of a familiar test. Pediatr Rev 2006; 27: 382-384.
10. Nauseef WM, Brigham S, Cogley M. Hereditary myeloperoxidase deficiency due to a missense mutation of arginine 569 to tryptophan. J Biol Chem 1994; 269: 1212-1216.
11. Nauseef WM, Cogley M, McCormick S. Effect of the R569W missense mutation on the biosynthesis of myeloperoxidase. J Biol Chem 1996; 271: 9546-9549.
12. Miyauchi J, Ohyashiki K, Inatomi Y, Toyama K. Neutrophil secondary-granule deficiency as a hallmark of all-trans retinoic acid-induced differentiation of acute promyelocytic leukemia cells. Blood 1997; 90: 803-813.
13. Zhu H, Yang L, Zhou B, Yu R, Tan N, Wang B. Myeloperoxidase G–463A polymorphism and the risk of gastric cancer: a case–control study. Carcinogenesis 2006; 27: 2491-2496.
14. Brennan ML, Penn MS, Van Lente F et al. Prognostic value of myeloperoxidase in patients with chest pain. N Engl J Med 2003; 349: 1595-1604.
15. Reynolds WF, Hiltunen M, Pirskanen M et al. MPO and APOE epsilon-4 polymorphisms interact to increase risk for AD in Finnish males. Neurology 2000; 55: 1284-1290.
16. Milacic S. Examining activity alkaline phosphatase and myeloperoxidase in the peripheral blood granulocytes due to ionizing radiations (Master's Thesis). University of Belgrade, Medical Faculty, 1990.
17. Moorhed PS. Chromosome preparation of leukocytes cultured from human peripheral blood. Exp Cell Res 1960; 20: 613-616.
18. Weil SC, Rosner GL, Reid MS et al. Translocation and rearrangement of myeloperoxidase gene in acute promyelocytic leukemia. Science 1988; 240: 790-792.
19. Kizaki M, Miller CW, Selsted ME, Koeffler HP. Myeloperoxidase (MPO) gene mutation in hereditary MPO deficiency. Blood 1994; 83: 1935-1940.
20. Knezević I, Milacic S, Novak Lj, Nesic V. The effect of $CaNa_2EDTA$ on excretion of ^{210}PB, ^{210}Po and stable lead in cases of chronic lead intoxication. Rad Protect Dosimetry 1998; 79: 471-472.
21. Milacic S. Enzymatic activity (MPO-Myeloperoxidase) in granulocytes in colored blood smears. Health Phys 2004; 86: 457-459.
22. Milacic S. Aberrations of genetic material as biomarkers of ionizing radiation effects. 11th International Congress of the International Radiation Protection Association, 22-28 May 2004, Madrid, Spain. Proceedings of the IRPA 11, published on CD (full papers) 2004; produced by SENDA, ISBN: 84-87078-05-2. Available online at www.irpa11.com
23. Atanasova P, Hadjidekova V, Agova S, Iovtchev M. Chromosomal aberrations in radiation waste repository workers detected by FISH painting and giemsa Staining. Turk J Med Sci 2004; 34: 359-365.
24. Sharma U, Sharma A, Sharma ML, Saxena S. Leukocyte alkaline phosphatase (LAP) activity in health and infections of infancy. Indian J Pediatr 1983; 50: 275-277.
25. Mrdjanovic J, Jakimov D, Tursijan S, Bogdanovic G. Evaluation of sister chromatid exchanges, micronuclei, and proliferating rate index in hospital workers chronically exposed to ionizing

radiation. J BUON 2005; 10: 99-103.
26. Boffetta P, Hel O, Norppa H et al. Chromosomal aberrations and cancer risk: results of a cohort study from central Europe. Am J Epidemiol 2007; 165: 36-43.
27. Bonassi S, Ugolini D, Kirsh-Volders M, Stromberg U, Vermeulen R, Tucker JD. Human population studies with cytogenetic biomarkers: Review of the literature and future prospective. Environ Mol Mutagen 2005; 45: 258-270.
28. Chobanova N, Vukov M, Yagova A. Cancer incidence among Bulgarian medical radiation workers: epidemiological study. J BUON 2007; 12: 65-69.
29. Milacic S. The incidence of malignant neoplasms in individuals working in areas of ionizing radiation in hospitals. J BUON 2008; 13: 377-384.