research article

Antioxidant defence-related genetic variants are not associated with higher risk of secondary thyroid cancer after treatment of malignancy in childhood or adolescence

Ana Lina Vodusek,[1] Katja Goricar,[2] Barbara Gazic,[3] Vita Dolzan,[2] Janez Jazbec[4]

[1] Department of Radiation Oncology, Institute of Oncology Ljubljana, Ljubljana, Slovenia
[2] Pharmacogenetics Laboratory, Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia
[3] Department of Pathology, Institute of Oncology Ljubljana, Ljubljana, Slovenia
[4] Department of Hematology and Oncology, University Children's Hospital, Ljubljana, Slovenia

Radiol Oncol 2016; 50(1): 80-86.

Received 2 February 2015
Accepted 23 February 2015

Correspondence to: Prof. Janez Jazbec, Ph.D., M.D., Department of Hematology and Oncology, University Children's Hospital, Bohoričeva 20, 1525 Ljubljana, Slovenia. Phone: +386 1 522 8653; Fax: +386 1 522 4036; E-mail: janez.jazbec@mf.uni-lj.si

Disclosure: No potential conflicts of interest were disclosed.

Background. Thyroid cancer is one of the most common secondary cancers after treatment of malignancy in childhood or adolescence. Thyroid gland is very sensitive to the carcinogenic effect of ionizing radiation, especially in children. Imbalance between pro- and anti-oxidant factors may play a role in thyroid carcinogenesis. Our study aimed to assess the relationship between genetic variability of antioxidant defence-related genes and the risk of secondary thyroid cancer after treatment of malignancy in childhood or adolescence.

Patients and methods. In a retrospective study, we compared patients with childhood or adolescence primary malignancy between 1960 and 2006 that developed a secondary thyroid cancer (cases) with patients (controls), with the same primary malignancy but did not develop any secondary cancer. They were matched for age, gender, primary diagnosis and treatment (especially radiotherapy) of primary malignancy. They were all genotyped for *SOD2* p.Ala16Val, *CAT* c.-262C>T, *GPX1* p.Pro200Leu, *GSTP1* p.Ile105Val, *GSTP1* p.Ala114Val and *GSTM1* and *GSTT1* deletions. The influence of polymorphisms on occurrence of secondary cancer was examined by McNemar test and Cox proportional hazards model.

Results. Between 1960 and 2006 a total of 2641 patients were diagnosed with primary malignancy before the age of 21 years in Slovenia. Among them 155 developed a secondary cancer, 28 of which were secondary thyroid cancers. No significant differences in the genotype frequency distribution were observed between cases and controls. Additionally we observed no significant influence of investigated polymorphisms on time to the development of secondary thyroid cancer.

Conclusions. We observed no association of polymorphisms in antioxidant genes with the risk for secondary thyroid cancer after treatment of malignancy in childhood or adolescence. However, thyroid cancer is one of the most common secondary cancers in patients treated for malignancy in childhood or adolescence and the lifelong follow up of these patients is of utmost importance.

Key words: secondary thyroid cancer; antioxidant genes; genetic polymorphism

Introduction

Modern treatment modalities and better diagnostic techniques greatly improved the survival of children and adolescents with malignancies.[1,2] With increasing number of survivors and years of follow up late effects of treatment are encountered more frequently.[3,4]

The most detrimental late effects are secondary cancers. Several studies report on increased risk of subsequent secondary cancers even several decades after treatment of primary malignancy.[5] Increased incidence of secondary thyroid cancer was reported even up to 40 years after radiotherapy.[6-9]

The thyroid gland is very sensitive to the carcinogenetic effect of ionizing radiation, especially in children.[7] Ionizing radiation damages DNA directly or indirectly through production of free radicals and reactive oxygen species (ROS). It has been shown that gamma radiation and hydrogen peroxide (H_2O_2), which is one of the ROS, induce similar DNA damages in the thyroid.[10] Oxidative DNA damage involves single- or double DNA strand breaks, purine and pyrimidine or deoxyribose modifications as well as DNA cross links. ROS can also damage the cell through lipid peroxidation, protein modification, membrane disruption and mitochondrial damage.[11,12]

The thyroid cell is constantly exposed to ROS and an imbalance between pro- and anti-oxidative factors has been suggested as an important mechanism in thyroid carcinogenesis. The accumulation of oxidative DNA damage may drive genomic instability events and lead to somatic mutations. Many studies have shown that oxidants are increased and antioxidants are decreased in patients with thyroid cancer.[13-19]

The most important antioxidants in the thyroid are antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT). Manganese superoxide dismutase (SOD2) is the major antioxidant in mitochondria, catalysing the dismutation of superoxide anion to H_2O_2, which is then reduced to water by CAT or GPX.[20,21] Many studies have investigated genetic variability in genes coding for antioxidant enzymes and their relationship to cancer risk, however the results were inconclusive[22] and the data on thyroid cancer risk are lacking.[23]

The most common polymorphism in the gene coding for SOD2 (*SOD2*) leads to substitution of alanine (Ala) with valine (Val) at codon 16 (p.Ala16Val) and affects transport of the enzyme into the mitochondria.[24] According to several studies the 16Ala allele of *SOD2* polymorphism is associated with an increased risk of prostate and oesophageal cancer.[21,25]

CAT activity is affected by functional single nucleotide polymorphism (SNP) *CAT* c.-262C>T in the promoter region of *CAT* gene, which leads to lower catalase activity. This polymorphism was implicated in increased susceptibility to breast and cervical cancer.[26,27]

The most common *GPX1* polymorphism results in the amino acid substitution of leucine with proline at codon 200 (p.Leu200Pro) and results in lower enzyme activation. As it may influence the balance between oxidative stress and antioxidant defence, it may therefore increase cancer risk.[28] Indeed several studies associate *GPX1* p.Pro200Leu polymorphism with increased susceptibility to prostate and breast cancer.[29,30]

Glutathione S-transferase (GSTs) enzymes, encoded by *GST* genes, are implicated in detoxification of xenobiotics and reactive products of ROS, so they may have a crucial role in protecting tissue from oxidative damage.[31] Deletions of the *GSTM1* and *GSTT1* genes result in null genotypes and lead to impaired enzyme activity.[32] In *GSTP1* two frequent SNPs resulting in an amino acid substitution have been reported. The *GSTP1* p.Ile105Val SNP results in Ile to Val substitution near the active site and leads to decreased enzyme activity. The functional role of the *GSTP1* p.Ala114Val polymorphism is not clear, however, reduced conjugation capacity was reported in the enzyme with both polymorphisms present.[30] Variants of these loci have been implicated in the aetiology of numerous cancers.[29,31-34]

There is some inconclusive data on *GST* polymorphisms in association to thyroid cancer risk. According to some studies individuals with homozygous deletions of *GSTM1* or *GSTT1* have an increased risk of thyroid cancer, whereas Lemos *et al*. found the opposite in his study.[33-35] Mertens *et al*. studied radiotherapy related malignancies in survivors of Hodgkin disease and found that individuals lacking *GSTM1* but not *GSTT1* were at increased risk of any subsequent SMN.[36] To our knowledge data on genetic variability of antioxidant enzymes in primary thyroid cancer are scarce, while no data have been published regarding the secondary thyroid cancer.

The aim of the present study was to investigate the relationship between genetic variability in antioxidant defence-related genes (*SOD2* p.Ala16Val, *CAT* c.-262C>T, *GPX1* p.Pro200Leu, *GSTM1* deletion, *GSTT1* deletion, *GSTP1* p.Ile105Val and *GSTP1* p.Ala114Val) and the risk of secondary thyroid cancer after treatment of malignancy in childhood or adolescence.

Patients and methods

Patients

A population based study of all patients known to have developed a secondary thyroid cancer after treatment of malignancy in childhood or adolescence was performed. A retrospective matched case-control study was designed. Individuals were eligible for inclusion in the study patients group (cases) if they were diagnosed with any kind of primary malignancy between 1960 and 2006 and before the age of 21 and were treated at the Department of Hematology and Oncology, University Children's Hospital, Ljubljana, or at the Institute of Oncology, Ljubljana and had a secondary thyroid cancer diagnosed 5 years or later after the primary malignancy. Study controls were patients with a primary malignancy in childhood or adolescence but free of secondary thyroid cancer. They were selected with a ratio of 1 control to 1 case matched for: type of the primary malignancy, treatment of primary malignancy, especially regarding the radiotherapy to the neck, head or mediastinal region, sex and age at the time of primary malignancy diagnosis (if possible not more than 2 years younger or older then the case). Study patients and study controls were identified from a search from the Cancer Registry of Slovenia.[37]

All patients with primary childhood or adolescent malignancy were followed up at the Department of Hematology and Oncology, University Children's Hospital, Ljubljana, or at the out-patient Clinic for Late Effects at the Institute of Oncology, Ljubljana.[38]

Thyroid follow-up included yearly thyroid stimulating hormone (TSH) and thyroglobulin level evaluation and occasional neck ultrasound. All patients with palpable nodules and/or elevated thyroglobulin levels underwent a neck ultrasound as a method commonly used in the work-up of thyroid diseases.[39] If malignancy was suspected, fine needle aspiration biopsy (FNAB) was performed. When papillary/follicular lesions were detected or were just suspected by cytology, thyroidectomy was performed at the Institute of Oncology, Ljubljana.

The follow up interval was defined as the time between primary malignancy and secondary thyroid cancer in the study group or between primary malignancy and the last appointment at the Out-Patient Clinic for Late Effects in the control group. All patient's data (demographic, clinical and treatment data) were collected from the patient's medical records.

Single experienced pathologist reviewed all the primary malignancies and corresponding thyroid cancers.

The study was approved by the Slovenian Ethics Committee for Research in Medicine (No.138/04/10) and was carried out according to the Declaration of Helsinki.

DNA isolation and genotyping

According to the presence of tumour or normal tissue on hematoxylin and eosin (HE) slides the pathologist chose one representative paraffin block from each biopsy and marked the tumour and normal tissue area on the block. From the marked area (if possible we chose normal tissue) two to three cores of 1 mm in diameter were obtained for DNA extraction using a QIAamp DNA Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.[40]

For two control patients genomic DNA was isolated from archived cytological smears of bone marrow specimens using the QIAamp DNA Mini kit (Qiagen, Hilden, Germany).[41] For all other patients DNA was obtained from paraffin blocks contained tumour or normal tissue as described above.

Genotypes of *GPX1* p.Pro200Leu (rs1050450) and *SOD2* p.Val16Ala (rs4880) were determined by TaqMan genotyping method (Applied Biosystems, Foster City, CA, USA) as described previously.[42] Genotyping of *CAT* c.-262C>T (rs1001179), *GSTP1* p.Ile105Val (rs1695) and *GSTP1* p.Ala114Val (rs1138272) was carried out using a fluorescence-based competitive allele-specific (KASPar) assay (KBiosciences, Herts, UK).[43]

Multiplex polymerase chain reaction (PCR) was used for detection of *GSTM1* and *GSTT1* gene deletions. *GSTM1*, *GSTT1*, and *BGLO* genes were simultaneously amplified in a multiplex PCR reaction as previously described.[41] This approach allowed us to identify homozygous *GSTM1* or *GSTT1* gene deletion, but we could not distinguish between carriers of one or two copies of each gene.

Statistical analysis

Frequencies were used to describe the distribution of categorical variables and median and interquartile ranges were used for continuous variables. Standard chi-square test was used to assess deviation from Hardy-Weinberg equilibrium (HWE), comparing the distribution of genotype frequencies in the control group with the expected distribution within the population.[44]

To compare the genotype distribution, McNemar test for the analysis of matched samples based on binomial distribution was used. The influence of polymorphisms on the time to the occurrence of second cancer was examined by Cox proportional hazards model with stratification on the matched pairs to calculate relative risks (RRs) and their 95% confidence intervals (CIs).

All statistical analyses were carried out by IBM SPSS Statistics, version 19.0 (IBM Corporation, Armonk, NY, USA), except for odds ratios (OR) and 95% CIs in McNemar test that were calculated using GraphPad Software. A dominant genetic model was used in all statistical analyses and p values below 0.05 were considered statistically significant.

Results

Patients' characteristics

Based on data from The Cancer Registry of Slovenia, in the period between 1960 and 2006 a total of 2641 patients were diagnosed with primary cancer before the age of 21 years.[40] Among them 155 developed secondary cancer (5.9%), out of which 28 (18.1%) were secondary thyroid cancers.

Only 24 (85.7%) eligible cases were included in the study because we could not get the histopathological material for 4 controls. Therefore the study group included 8 (33.3%) males and 16 (66.7%) females with a median age of 12.7 years at diagnosis of primary cancer (range 7.1–6.7 years) and 29.2 years at diagnosis of secondary thyroid cancer (range 23.5–35.4 years). Six out of 24 patients (25.0%) were under 5 years old at the time of primary diagnosis. The control group included 8 (33.3%) males and 16 (66.7%) females with a median age of 12.9 years at diagnosis of primary cancer (range 5.0–15.4 years).

The most frequent primary cancer was Hodgkin's disease (HD) (15 pairs, 62.5%), then acute lymphoblastic leukemia (ALL) (2 pairs, 8.3%) and central nervous system (CNS) tumours (2 pairs, 8.3%). Non-Hodgkin lymphoma (NHL), neuroblastoma, rhabdomyosarcoma, nasopharyngeal carcinoma and ovarian tumours were observed in 1 pair each (4.2%). Most of the patients with secondary thyroid cancer received radiation therapy to the head, neck or mediastinum during the treatment for primary cancer (23 patients, 95.8%): 15 (62.5%) to the neck, 6 (25.0%) to the head and 2 (8.3%) to the mediastinum. The same distribution of irradiated sites was observed also in the control group.

The most frequent histology of secondary thyroid cancer was papillary carcinoma (23, 95.8%). Only 1 tumour (4.2%) was follicular neoplasm of undefined malignant potential. Using TNM classification for staging most of thyroid cancer were stage 1 with tumour localised to the thyroid and/or lymph nodes (23, 95.8%), 1 (4.2%) was stage 2 with lung metastasis (M1). Among 12 (50.0%) T1 tumours (tumour diameter ≤ 2cm), there were 9 (37.5%) microcarcinoma (T1a: tumour ≤ 10 mm). There were 2 (8.3%) T2 tumours (tumour > 2 cm but ≤ 4 cm in greatest dimension, limited to the thyroid); 6 (25.0%) were T3 (minimal extrathyroid extension) and 3 (12.5%) were T4 (extending beyond the thyroid capsule to invade subcutaneous soft tissues, larynx, trachea, oesophagus, or recurrent laryngeal nerve) and 11 (45.8%) had regional lymph node metastasis (N1).

A total or near total thyroidectomy was carried out in all cases. Additional radioiodine treatment was applied to 19 (79.2%) patients. In 6 (25.0%) cases lymph node metastases were excised.

The follow up interval was comparable in both groups and was 19.6 (range 9.0–23.6) years in the primary group and 18.8 (range 12.80–27.6) years in the control group. Both groups did not differ significantly regarding the demographic data.

Genotype frequencies of the antioxidant defence-related genes are presented in the Table 1. All the investigated polymorphisms were in HWE in the control group.

To assess if the investigated polymorphisms influence the risk of secondary thyroid cancer, we performed a matched analysis. When all the cases were compared to controls, no significant differences in the genotype frequency distribution were observed (Table 2). There were also no differences in genotype distribution between microcarcinoma and other secondary thyroid cancers.

We also assessed the influence of investigated polymorphisms on time to development of secondary thyroid cancer using Cox regression with stratification on matched pairs. We have not observed any association between studied polymorphisms and the time pattern of occurrence of secondary thyroid cancers after treatment of malignancy in childhood or adolescence.

Discussion

In the present study we investigated if *SOD2* p.Ala16Val, *CAT* c.-262C>T, *GPX1* p.Pro200Leu, *GSTM1*, *GSTT1*, *GSTP1* p.Ile105Val and *GSTP1* p.Ala114Val polymorphisms influence the risk of secondary thyroid cancer after treatment of malig-

TABLE 1. Genotype frequencies of the antioxidant defence-related genes

SNP	Genotype	All patients N (%)	Cases N (%)	Controls N (%)	P_{HWE} controls
GPX1 rs1050450 p. Pro200Leu	CC	28 (58.3)	16 (66.7)	12 (50)	0.967
	CT	17 (35.4)	7 (29.2)	10 (41.7)	
	TT	3 (6.3)	1 (4.2)	2 (8.3)	
SOD2[a] rs4880 p.Val16Ala	GG	10 (21.7)	4 (16.7)	6 (27.3)	0.338
	GA	31 (67.4)	18 (75)	13 (59.1)	
	AA	5 (10.9)	2 (8.3)	3 (13.6)	
CAT rs1001179 c.-262G>A	GG	32 (66.7)	16 (66.7)	16 (66.7)	0.834
	GA	14 (29.2)	7 (29.2)	7 (29.2)	
	AA	2 (4.2)	1 (4.2)	1 (4.2)	
GSTP1 rs1695 p.Ile105Val	AA	22 (45.8)	10 (41.7)	12 (50)	0.432
	AG	23 (47.9)	12 (50)	11 (45.8)	
	GG	3 (6.3)	2 (8.3)	1 (4.2)	
GSTP1 rs1138272 p.Ala114Val	CC	39 (81.3)	19 (79.2)	20 (83.3)	0.106
	CT	8 (16.7)	5 (20.8)	3 (12.5)	
	TT	1 (2.1)	/	1 (4.2)	
GSTM1[b] gene deletion	non-null	23 (48.9)	14 (58.3)	9 (39.1)	
	null	24 (51.1)	10 (41.7)	14 (60.9)	
GSTT1[b] gene deletion	non-null	39 (83)	19 (79.2)	20 (87)	
	null	8 (17)	5 (20.8)	3 (13)	

CAT = catalase; *GPX* = glutathione peroxidase; *GSTM1* = glutathione S-transferase Mu 1; *GSTP1*= glutathione S-transferase pi gene; *GSTT1* = glutathione S-transferase theta 1; HWE= Hardy-Weinberg equilibrium; N = number; SNP = single nucleotide polymorphism; SOD2 = manganese superoxide dismutase; [a]data missing for 2 controls; [b]data missing for 1 control

nancy in childhood or adolescence. To the best of our knowledge no such study was yet performed in the secondary thyroid cancer, while data on the role of common functional polymorphisms in antioxidant defence-related genes in the primary thyroid cancer are scarce.[29]

In total 5.9% of patients diagnosed between 1960 and 2006 with primary malignancy before the age of 21 years has developed a secondary cancer. Secondary thyroid cancers represented 18% of secondary cancers. Our data are comparable to other studies and show that thyroid cancer is one of the most common secondary cancers after treatment of malignancy in childhood or adolescence. Similar to other studies the most frequent primary malignancy was Hodgkin's lymphoma and the median time to develop a secondary thyroid cancer was 19.6 years.[2-6]

According to several studies the *SOD2* 16Ala allele was associated with an increased risk of prostate and oesophageal cancer, but with decreased risk of lung cancer.[21,24] Never the less, the meta-analysis showed no significant effect of *SOD2* p.Val16Ala polymorphism on overall cancer risk.[21] This is also in concordance with our data that show no association between the *SOD2* polymorphism and the risk of secondary thyroid cancer.

TABLE 2. Influence of selected polymorphisms on the risk for secondary thyroid cancer

SNP	OR (95% CI)	p
GPX1 (rs1050450)	0.43 (0.07-1.88)	0.344
SOD2[a] (rs4880)	1.50 (0.36-7.23)	0.754
CAT (rs1001179)	1.00 (0.27-3.74)	1.000
GSTP1 (rs1695)	1.40 (0.38-5.59)	0.774
GSTP1 (rs1138272)	1.25 (0.27-6.30)	1.000
GSTM1 (gene deletion)[b]	0.43 (0.07-1.88)	0.344
GSTT1 (gene deletion)[b]	2.00 (0.29-22.11)	0.687

CAT = catalase; CI = confident interval; *GPX* = glutathione peroxidase; *GSTM1* = glutathione S-transferase Mu 1; *GSTP1*= glutathione S-transferase pi gene; *GSTT1* = glutathione S-transferase theta 1; OR = odd ratio; SNP = single nucleotide polymorphism; *SOD2* = manganese superoxide dismutase; [a]data missing for 2 controls; [b]data missing for 1 control

Recent studies suggested that the *GPX1* p.Pro200Leu polymorphism increased the susceptibility to bladder cancer[27], whereas a meta-analysis showed no significant association of *GPX1* p.Pro200Leu polymorphism with cancer risk in general.[45] Similar to the meta-analysis we observed no association between *GPX1* p.Pro200Leu polymorphism and the risk of secondary thyroid cancer.

CAT polymorphism was implicated in cancerogenesis of several tumours, including breast and cervical cancer[24], but again, meta-analysis has not confirmed these observations for the breast cancer risk.[46] Our results also showed no association between *CAT* polymorphism and risk of secondary thyroid cancer.

Genetic variability at the *GSTM1, GSTT1 and GSTP1* loci has been linked to increased susceptibility to several cancers, including thyroid cancer.[28,29,31] Our study was the first to analyse the association between the *GST* polymorphisms (*GSTM1, GSTT1, GSTP1* p.Ile105Val and *GSTP1* p.Ala114Val) and the risk of developing secondary thyroid cancer after treatment of malignancy in childhood or adolescence, however, we were not able to detect any significant association. Mertens *et al*. found only a non-significantly increased risk of thyroid cancer in *GSTM1* or *GSTT1* homozygous patients that had Hodgkin lymphoma as a primary cancer[36], but a meta-analysis concluded that GST polymorphisms are unlikely to be major determinants of susceptibility to primary thyroid cancer.[29]

Our results are comparable to the results of relevant studies on antioxidant defence-related polymorphisms and the risk of cancer in general. While the sample size is small and therefore in some instances lacks statistical power, its prime advantage is the homogeneity of data because we designed a population-based study, which included all patients diagnosed and treated for secondary thyroid cancer in Slovenia after treatment of malignancy in childhood or adolescence between 1960 and 2006.

In conclusion, we observed no association of common functional polymorphisms in antioxidant defence related genes with the risk for secondary thyroid cancer after treatment of malignancy in childhood or adolescence. However, thyroid cancer is one of the most common secondary cancers after treatment of malignancy in childhood or adolescence and it can develop several decades after the treatment. Hence the lifelong follow up of patients with childhood or adolescent malignancy is of utmost importance and further studies on genetic factors associated with thyroid cancer risk should be performed.

Acknowledgements

The authors thank Matej Kastelic, Ph.D. for the help with the laboratory analyses, Maruša Debeljak, Ph.D. for help with DNA isolation from archived cytological smears of bone marrow specimens and prof. Berta Jereb, Ph.D., M.D. for all the support.

This work was financially supported by the Infrastructure program for the Lifelong follow-up of the survivals from childhood or adolescent cancer (Grant No. I0 - 0010) and by the Slovenian Research Agency (ARRS Grant No.P1-0170).

References

1. Perme MP, Jereb B. Trends in survival after childhood cancer in Slovenia between 1957 and 2007. *Pediatr Hematol Oncol* 2009; **26:** 240-51.
2. Kachanov DY, Dobrenkov KV, Shamanskaya TV, Abdullaev RT, Inushkina EV, Savkova1 RF, et al. Solid tumors in young children in Moscow Region of Russian Federation. *Radiol Oncol* 2008; **42:** 39-44.
3. Jazbec J, Ećimović P, Jereb B. Second neoplasms after treatment of childhood cancer in Slovenia. *Pediatr Blood Cancer* 2004; **42:** 574-81.
4. Zaletel L, Bratanic N, Jereb B. Gonadal function in patients treated for Hodgkin's disease in childhood. *Radiol Oncol* 2010; **44:** 87-193.
5. Doi K, Mieno MN, Shimada Y, Yonehara H, Yoshinaga S. Methodological extensions of meta-analysis with excess relative risk estimates: application to risk of second malignant neoplasms among childhood cancer survivors treated with radiotherapy. *J Radiat Res* 2014; **55:** 885-901.
6. Bhatti P, Veiga LH, Ronckers CM, Sigurdson AJ, Stovall M, Smith SA, et al. Risk of second primary thyroid cancer after radiotherapy for a childhood cancer in a large cohort study: an update from the childhood cancer survivor study. *Radiat Res* 2010; **174:** 741-52
7. Sigurdson AJ, Ronckers CM, Mertens AC, Stovall M, Smith SA, Liu Y, et al. Primary thyroid cancer after a first tumour in childhood (the Childhood Cancer Survivor Study): a nested case-control study. *Lancet* 2005; **365:** 2014-23.
8. Sadetzki S, Chetrit A, Lubina A, Stovall M, Novikov I. Risk of thyroid cancer after childhood exposure to ionizing radiation for tinea capitis. *J Clin Endocrinol Metab* 2006; **91:** 4798-804.
9. Acharya S, Sarafoglou K, LaQuaglia M, Lindsley S, Gerald W, Wollner N, et al. Thyroid neoplasms after therapeutic radiation for malignancies during childhood or adolescence. *Cancer* 2003; **97:** 2397-403.
10. Versteyhe S, Driessens N, Ghaddhab C, Tarabichi M, Hoste C, Dumont JE, et al. Comparative analysis of the thyrocytes and T cells: responses to H2O2 and radiation reveals an H2O2-induced antioxidant transcriptional program in thyrocytes. *J Clin Endocrinol Metab* 2013; **98:** E1645-54.
11. van Loon B, Markkanen E, Hübscher U. Oxygen as a friend and enemy: how to combat the mutational potential of 8-oxo-guanine. *DNA Repair* 2010; **9:** 604-16.
12. Karger S, Krause K, Engelhardt C, Weidinger C, Gimm O, Dralle H, et al. Distinct pattern of oxidative DNA damage and DNA repair in follicular thyroid tumours. *J Mol Endocrinol* 2012; **48:** 193-202.
13. Erdamar H, Cimen B, Gülcemal H, Saraymen R, Yerer B, Demirci H. Increased lipid peroxidation and impaired enzymatic antioxidant defence mechanism in thyroid tissue with multinodular goiter and papillary carcinoma. *Clin Biochem* 2010; **43:** 650-4.
14. Krohn K, Maier J, Paschke R. Mechanisms of disease: hydrogen peroxide, DNA damage and mutagenesis in the development of thyroid tumors. *Nat Clin Pract Endocrinol Metab* 2007; **3:** 713-20.
15. Karbownik-Lewińska M, Kokoszko-Bilska A. Oxidative damage to macromolecules in the thyroid - experimental evidence. *Thyroid Res* 2012; **5:** 25.

16. Xing M. Oxidative stress: a new risk factor for thyroid cancer. *Endocr Relat Cancer* 2012; **19:** 7-11.

17. Young O, Crotty T, O'Connell R, O'Sullivan J, Curran AJ. Levels of oxidative damage and lipid peroxidation in thyroid neoplasia. *Head Neck* 2010; **32:** 750-6.

18. Wang D, Feng JF, Zeng P, Yang YH, Luo J, Yang YW. Total oxidant/antioxidant status in sera of patients with thyroid cancers. *Endocr Relat Cancer* 2011; **18:** 773-82.

19. Akinci M, Kosova F, Cetin B, Sepici A, Altan N, Aslan S, et al. Oxidant/antioxidant balance in patients with thyroid cancer. *Acta Cir Bras* 2008; **23:** 551-4.

20. Banescu C, Trifa AP, Voidazan S, Moldovan VG, Macarie I, Benedek Lazar E, et al. CAT, GPX1, MnSOD, GSTM1, GSTT1, and GSTP1 genetic polymorphisms in chronic myeloid leukemia: a case-control study. *Oxid Med Cell Longev* 2014; 2014: 875861.

21. Wang S, Wang F, Shi X, Dai J, Peng Y, Guo X, et al. Association between manganese superoxide dismutase (MnSOD) Val-9Ala polymorphism and cancer risk - A meta-analysis. *Eur J Cancer*.2009; **45:** 2874-81.

22. Janicka A, Szymańska-Pasternak J, Bober J. [Polymorphisms in the oxidative stress-related genes and cancer risk]. [Article in Polish]. *Ann Acad Med Stetin* 2013; **59:** 18-28.

23. Lin JC, Kuo WR, Chiang FY, Hsiao PJ, Lee KW, Wu CW, et al. Glutathione peroxidase 3 gene polymorphisms and risk of differentiated thyroid cancer. *Surgery* 2009; **145:** 508-13.

24. Sutton A, Imbert A, Igoudjil A, Descatoire V, Cazanave S, Pessayre D, et al. The manganese superoxide dismutase Ala16Val dimorphism modulates both mitochondrial import and mRNA stability. *Pharmacogenet Genomics* 2005; **15:** 311-9.

25. Sun GG, Wang YD, Lu YF, Hu WN. Different association of manganese superoxide dismutase gene polymorphisms with risk of prostate, esophageal, and lung cancers: evidence from a meta-analysis of 20,025 subjects. *Asian Pac J Cancer Prev* 2013; **14:** 1937-43.

26. Castaldo SA, da Silva AP, Matos A, Inácio A, Bicho M, Medeiros R, et al. The role of CYBA (p22phox) and catalase genetic polymorphisms and their possible epistatic interaction in cervical cancer. *Tumour Biol* 2015; **36:** 909-14.

27. Geybels MS, van den Brandt PA, van Schooten FJ, Verhage BA. Oxidative stress-related genetic variants, pro- and antioxidant intake and status, and advanced prostate cancer risk. *Cancer Epidemiol Biomarkers Prev* 2015; **24:** 178-86.

28. Hong Z, Tian C, Zhang X. GPX1 gene Pro200Leu polymorphism, erythrocyte GPX activity and cancer risk. *Mol Biol Rep* 2013; **40:** 1801-12.

29. Li J, Long J, Hu Y, Tan A, Guo X, Zhang S. Glutathione S-transferase M1, T1, and P1 polymorphisms and thyroid cancer risk: a meta-analysis. *Cancer Epidemiol* 2012; **36:** 333-40.

30. Bohanec Grabar P, Logar D, Tomsic M, Rozman B, Dolzan V. Genetic polymorphisms of glutathione S-transferases and disease activity of rheumatoid arthritis. *Clin Exp Rheumatol* 2009; **27:** 229-36.

31. Zhuo X, Cai L, Xiang Z, Li Q, Zhang X. GSTM1 and GSTT1 polymorphisms and nasopharyngeal cancer risk: an evidence-based meta-analysis. *J Exp Clin Cancer Res* 2009; **28:** 46.

32. Koh WP, Nelson HH, Yuan JM, Van den Berg D, Jin A, Wang R, et al. Glutathione S-transferase (GST) gene polymorphisms, cigarette smoking and colorectal cancer risk among Chinese in Singapore. *Carcinogenesis* 2011; **32:** 1507-11.

33. Ho T, Zhao C, Zheng R, Liu Z, Wei Q, Sturgis EM. Glutathione S-transferase polymorphisms and risk of differentiated thyroid carcinomas. A case control study. *Arch Otolaryngol Head Neck Surg* 2006; **132:** 756-61.

34. Lemos MC, Coutinho E, Gomes L, Carrilho F, Rodrigues F, Regateiro FJ, et al. Combined GSTM1 and GSTT1 null genotypes are associated with a lower risk of papillary thyroid cancer. *J Endocrinol Invest* 2008; **31:** 542-5.

35. Gaspar J, Rodrigues S, Gil OM, Manita I, Ferreira TC, Limbert E, et al. Combined effects of glutathione S-transferase polymorphisms and thyroid cancer risk. *Cancer Genet Cytogenet* 2004; **151:** 60-7.

36. Mertens AC, Mitby PA, Radloff G, Jones IM, Perentesis J, Kiffmeyer WR, et al. XRCC1 and glutathione-S-transferase gene polymorphisms and susceptibility to radiotherapy-related malignancies in survivors of Hodgkin disease. *Cancer* 2004; **101:** 1463-72.

37. *Cancer in Slovenia 2010*. Ljubljana: Institute of Oncology Ljubljana, Epidemiology and Cancer Registry, Cancer registry of Republic of Slovenia; 2013.

38. Jereb B. Model for long-term follow-up of survivors of childhood cancer. *Med Pediatr Oncol* 2000; **34:** 256-8.

39. Tatar IG, Kurt A, Yilmaz KB, Doğan M, Hekimoglu B, Hucumenoglu S. The role of elastosonography, gray-scale and colour flow Doppler sonography in prediction of malignancy in thyroid nodules. *Radiol Oncol* 2014; **48**: 348-53.

40. Goricar K, Kovac V, Jazbec J, Lamovec J, Dolzan V. Homologous recombination repair polymorphisms and the risk for osteosarcoma. *J Med Biochem* 2014; **33:** 1-8.

41. Jazbec J, Aplenc R, Dolzan V, Debeljak M, Jereb B. GST polymorphisms and occurrence of second neoplasms after treatment of childhood leukemia. *Leukemia* 2003; **17:** 2540-2.

42. Erculj N, Zadel M, Dolzan V. Genetic polymorphisms in base excision repair in healthy slovenian population and their influence on DNA damage. *Acta Chim Slov* 2010; **57:** 182-8.

43. Goricar K, Erculj N, Zadel M, Dolzan V. Genetic polymorphisms in homologous recombination repair genes in healthy Slovenian population and their influence on DNA damage. *Radiol Oncol* 2012; **46:**46-53.

44. Guo S, Thompson E. Performing the exact test of Hardy–Weinberg proportion for multiple alleles. *Biometrics* 1992; **48:** 361-72.

45. Cao M, Mu X, Jiang C, Yang G, Chen H, Xue W. Single-nucleotide polymorphisms of GPX1 and MnSOD and susceptibility to bladder cancer: a systematic review and meta-analysis. *Tumour Biol* 2014; **35:** 759-64.

46. Saadat M, Saadat S. Genetic polymorphism of CAT C-262 T and susceptibility to breast cancer, a case-control study and meta-analysis of the literature. *Pathol Oncol Res* 2015; **21:** 433-7.