Chirurgia (2015) 110: 254-261
No. 3, May - June

GSTM1, GSTT1 and GSTP1 Genetic Variants in Multiple Urologic Cancers

D. N. Chirilă[1], R. Popp[2*], Ş. Vesa[3*], O. Bălăcescu[4], I. Coman[5], N. A. Constantea[1], C. Ciuce[6]

[1]V[th] Surgical Clinic, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania
[2]Department of Genetics, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania
[3]Department of Pharmacology "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania
[4]Functional Genomics, Proteomics and Experimental Pathology Laboratory, The Oncology Institute „Ion Chiricută" Cluj-Napoca, Romania
[5]Department of Urology "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania
[6]1[st] Surgical Clinic, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

Rezumat

Variante genice ale GSTM1, GSTT1 și GSTP1 în cancerele urologice multiple

Introducere: Glutation S-transferazele (GST) sunt enzime de faza a II-a, răspunzătoare de catalizarea biotransformării unei mari varietăți de compuşi electrofili având un rol central în detoxifierea metaboliților activați ai procarcinogenilor produşi prin reacțiile de faza I, prin legarea lor de glutation şi favorizarea excreției acestora prin urină.
Obiective: am evaluat genotipurile GSTM1, GSTT1 şi GSTP1 la pacienții pacientele diagnosticați cu tumori maligne multiple, dintre care cel puțin una a fost localizată la nivelul prostatei, vezicii urinare sau rinichiului.
Material şi metodă: am evaluat genetic pentru genotipurile GSTM1, GSTT1 şi GSTP1 34 pacienți cu cancere multiple urologice şi 23 pacienți cu asocierea unui cancer urologic cu un alt tip de cancer. Rezultate: în lotul pacienților cu cancere urologice multiple genotipul nul GSTT1 a fost constatat la 26,4% dintre pacienți, comparativ cu 0% la martori; cel puțin un genotip nul GSTM1 sau GSTT1 au avut 82,35% dintre pacienți comparativ cu 47% martori; în lotul cu cancere diferite genotipul nul GSTM1 a fost constatat la 52,1% dintre pacienți, comparativ cu 4,3% la martori; genotipul nul GSTT1 a fost găsit la 34,7% dintre pacienți, comparativ cu 4,3% dintre martori; cel puțin un genotip nul GSTm1 sau GSTT1 a fost constatat la 73,9%, comparativ cu 8,6% în rândul martorilor.
Concluzii: Genotipul nul GSTT1 este un factor de risc pentru pacienții având mai multe tumori maligne primitive urologice (de vezică urinară, prostată şi rinichi); genotipul nul GSTM1 sau GSTT1 este mai frecvent întâlnit la pacienții cu neoplazii multiple urologice; genotipul nul GSTM1 şi GSTT1 reprezintă factori de risc pentru pacienții cu tipuri diferite de cancer, cel puțin unul fiind în sfera urinară.

Cuvinte cheie: cancere multiple, cancere urologice, GSTM1, GSTT1, GSTP1

Abstract

Introduction: Glutathione S-transferases (GSTs) are phase 2 enzymes responsible for catalyzing the biotransformation of a wide variety of electrophilic compounds, having a crucial role in the detoxification of active metabolites of procarcinogens produced by phase 1 reactions, tying them to glutathione and promoting their excretion in the urine.
Objectives: we evaluated GSTM1, GSTT1 and GSTP1 genotypes in patients diagnosed with multiple malignancies, of which at least one was found in the prostate, bladder or kidney.

Corresponding author: Daciana N. Chirila, MD, PhD
"Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca
Vth Surgical Clinic
11 Tabacarilor Street, Cluj-Napoca
code 400139, Romania
E-mail: dacianachirila@gmail.com

*author having an equal contribution as the first author

Materials and methods: GSTM1, GSTT1 and GSTP1 genotypes were genetically assessed in 34 patients with multiple urologic cancers and 23 patients with urologic cancer associated with another type of cancer.
Results: in the group of patients with multiple urologic cancers, GSTT1 null genotype was found in 26.4% of patients compared to 0% in controls, 82.35 % of patients and 47% of witnesses carried at least one GSTM1 or GSTT1 null genotype, and in the group with different cancers, GSTM1 null genotype was found in 52.1% of patients compared to 4.3% witnesses in the control group; GSTT1 null genotype was found in 34.7% of patients compared to 4.3% of witnesses, at least one GSTM1 or GSTT1 null genotype was found in 73.9% of patients compared to 8.6% of controls.
Conclusions: GSTT1 null genotype is a risk factor for patients with more primitive urologic malignancies (bladder, prostate and kidney); GSTM1 or GSTT1 null genotype is more frequent in patients with multiple urologic tumors; GSTM1 and GSTT1 null genotypes are risk factors in patients with different types of cancer, with at least one affecting the urinary system.

Key words: multiple cancers, urologic cancers, GSTM1, GSTT1, GSTP1

Introduction

The incidence of cancers in the urinary system has increased in recent years, particularly prostate cancer, which is the second most frequently diagnosed cancer in the world. Careful medical and urologic supervision, as well as modern means of investigation for these malignancies, allow for the diagnosis of a second cancer, often found in the urinary system as well, probably developing due to the existence of common risk factors, including genetic predisposition, or to the multifocal nature of transitional cell carcinomas, which are predominant in kidney and bladder cancers.

Working hypothesis. Objectives

The increased number of patients with multiple primitive urologic tumors diagnosed either simultaneously or at a variable interval of time, synchronous and metachronous, triggered the question of whether they have a connection with possible genetic changes in the three GSTs, known in the literature as being associated with various forms of cancers, including bladder, prostate and kidney cancers. We examined these patients with multiple cancers in comparison with a witness group to see if there are significant differences in GSTM1, GSTT1 and GSTP1 genetic variants.

Materials and methods

The study regarded patients diagnosed between 2005 and 2012 in Cluj-Napoca County Clinic Hospital with several distinct primitive cancers, of which at least one was found in the bladder, prostate or kidney. We studied GSTM1, GSTT1 and GSTP1 genetic variants in patients identified with multiple urologic malignancies and in those who have been diagnosed with a urologic cancer associated with another type of cancer. The total number of patients with urologic pathology (1), among which were chosen mostly those with multiple primitive cancers, was: 923 (18.44%) patients with prostate cancer, 868 (17.34%) patients with bladder cancer, 454 (9.07%) patients with renal cancer, 112 (2.23 %) patients with testicular cancer, 195 (3.89%) patients with history of cancer found in the urinary system. Thirty-six patients (16.58%[§]) had at least two distinct urologic malignancies and other 34 patients (15.66%) had a combination of urologic cancer and another cancer with a different location. The genetic study was conducted on 34 patients of the 36 identified with at least two distinct urologic malignant tumors, as two of them expressed their rejection. Of the 34 patients with various cancers, of which only one was found in the urinary system, 23 were analyzed, 5 patients had broken genetic material (obtained by DNA extraction from paraffin embedded biopsy samples) and some of them refused peripheral blood sampling (6 patients). The results of the genetic analysis of the two groups thus formed were compared with those obtained by genetic material processing in two corresponding groups of healthy controls, similar in age.

Patient clinical data were obtained from clinical observation sheets. Inclusion criteria in the study were: cases with at least two histologically verified primitive malignant tumors, of which at least one was found in the urinary system, regardless of the time of diagnosis (simultaneous, synchronous or metachronous); when there was clear histopathologic evidence of index tumor (in some cases being diagnosed many years before) we took into account the existence of adjuvant therapies, such as radiotherapy and chemotherapy, as factor supporting the diagnosis of malignancy. Exclusion criteria were the following: cases where more distinct malignant tumors of the bladder were diagnosed, sometimes dozens, knowing their multicentricity; cases where metastasis could not be excluded, even when three apparently distinct malignant tumors were present, found in three urinary organs, but having the same histopathology.

Patients included in the study signed their informed consent. There have also been cases (for patients who died) when the patient's preoperatively signed consent was used, which implied acceptance on the use of data and materials collected as anonymous. The genetic material was obtained by sampling 2 ml of peripheral blood or by paraffin-embedded biopsy samples. Genetic analysis consisted of genotyping GSTM1, GSTT1 and GSTP1, comparing the groups included

[§]The figures in parentheses represent the percentage of patients diagnosed with at least two primitive malignant tumors found in the urinary system, out of the 217 cases of patients diagnosed with multiple primitive cancers throughout the 7 ½-year period.

in the study with an adequate number of healthy controls with similar age, using chi-square test for statistical analysis.

We extracted the DNA of all the patients and controls from a quantity of 2 ml peripheral blood (on EDTA) or from paraffin embedded sample. This study was approved by Clinic County Hospital Ethic Board. For the DNA extraction from paraffin embedded samples we used a QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) based on protocol provided by the manufacturer. We used xylene in order to remove the paraffin, then ethanol for rehydrating the samples, then the incubation with proteinase K, purified on QIAamp MinElute column and eluted in nuclease free water; we measured the DNA concentrations with a Nanodrop spectrophotometer (NanoDrop Technologies; Wilmington, DE).

Some patients had a fragmented DNA which made impossible the search for genetic polymorphisms.

The absolute absence of the specific enzymes implicated in metabolic detoxification of carcinogens results in null GSTM1 and GSTT1 genotypes, and for the GSTP1 Ile105Val polymorphism. The genetic polymorphisms of GSTM1 and GSTT1 were determined simultaneously using a multiplex PCR protocol (2). We could not make the difference between heterozygous (+/0) and homozygous (+/+) genotype (both allele or only one allele without mutation), being able to determine null genotypes (-/-). We used three different primers for the amplification of both GSTM1 and GSTT1 allele (FwM1 5'-GAACTC CCTGAAAAGCTAAAGC-3'; RevM1 5'- GTTGGGCTCAAATATAGGGTGG - 3'and FwT1 5'-TTCCTTACT GGTCCTCACATCTC-3'; RevT1 5'- TCACCGGATCATGGC CAGCA-3'), also using an internal control, the amplification of β Globin. The gradient thermocycler (MastercyclerGradient, Eppendorf®, Germany) was used for PCR reactions. We obtained a total amount of aproximately 100 ng of genomic DNA, amplified then in a total volume of 25 μl reaction mixture containing 12.5 μl 2xPCR Master Mix (Fermentas MBI, Lituania®), 1 μl BSA (Bovine Serum Albumine, Fermentas MBI, Lituania ®) solution 5 mg/ml, 8 pM of each primer, forward and reverse (Eurogentec, Belgium®) and water free of nucleases to complete the 25 μl reaction volume. For analysing the amplification products we used electrophoresis in MetaPhor agarose gel (Lonza®, Basel, Switzerland). The null genotypes were found in the absence of amplification products. For the GSTP1 polymorphism we used a Wizard Genomic DNA Purification Kit (Promega®, MA, USA). The DNA was extracted from 300 μl of blood (leucocytes). The searched polymorphism of GSTP1 was analyzed by the polymerase chain reaction (PCR)–restriction fragment length polymorphism (RFLP) technique as it was described by Harries et al, 1997, with some modifications (3). The primers pair were 105F (5'-ACCCCAGGGCTCTATGGGAA-3') and 105R (5'-TGAGGGCACAAGAAGCCCCT-3'),12.5 μl 2xPCR Master Mix (Fermentas MBI, Lituania®), 1 μl BSA (Bovine Serum Albumine, Fermentas MBI, Lituania ®) solution 2 mg/ml, 8 pM of each primer, forward and reverse (Eurogentec, Belgium®) and water free of nucleases to complete the 25 μl volume for the amplification of the DNA. We used a gradient thermocycler (MastercyclerGradient, Eppendorf®) for the PCR reactions. The amplified fragment of 176 bp, result of the PCR reaction, was digested with 5 U BsmAI (Fermentas MBI, Lituania®); then we separated the fragments on a 3.0% Metaphor® agarose gel (Lonza®, Basel, Switzerland), and visualized them in a UV transilluminator (VilberLourmat Imaging System®, Marne-la-Vallée, France) after staining with ethidium bromide. We obtained three fragments of 176, 91 and 85 bp (the Ile/Val genotype), two fragments of 91 and 85 bp (a Val/Val homozygous genotype), or an undigested product (4) of 176 bp (an Ile/Ile genotype).

Results

Among the 34 patients, 14 presented an association of bladder cancer with prostate cancer, 10 had both bladder cancer and kidney cancer, 8 had both prostate cancer and kidney cancer, and 2 had bilateral renal cancer. The most common urologic index tumor was prostate cancer, followed by kidney and bladder cancer, respectively. In 11 cases, the tumors were synchronous. The second distinct primitive malignant tumor appeared after an average period of about 5 years. Of the 23 patients with malignant tumors in the urinary system, associated with other primitive cancers, 11 had kidney cancer, 8 bladder cancer and 4 had prostate cancer. The other primitive malignant tumors were: 8 colorectal cancers, 5 lymphomas, 4 breast cancers, 3 malignant melanomas, one gastric cancer, one laryngeal cancer, and one non melanoma skin cancer.

We examined 34 patients with two neoplasms in the urinary system in comparison with 34 controls with similar age ($\pm$2 years) to that of cancer patients. The average age at the occurrence of the second neoplasm in the urinary system was 68.8 $\pm$ 9.02 years (ranging between 48 and 87 years). The average age in the witness group was 67.46 $\pm$ 11.39 years (ranging between 48 and 85 years). The average age at first cancer diagnosis was 64.5 $\pm$ 9.9 years (ranging between 41 and 84 years). The ages of patients and witnesses had a normal distribution (Kolomorov-Smirnov test). There were no statistically significant differences between the average age of patients at study enrollment and that of controls (t test for independent variables, $p=0.5$). The location of the first cancer was in the prostate for 11 patients (32.3%), kidney for 12 patients (35.2%) and in the bladder for 11 patients (32.3%). The location of the second cancer was in the prostate for 10 patients (29.4%), kidney for 9 patients (26.4%) and in the bladder for 15 patients (44.1%). There were 19 smokers in the case group (55.8%) and 16 smokers in the control group (47.9%), without any statistically significant difference (chi-square test, $p=0.6$). GSTM1 null allele was found in 23 patients (67.6%) and in 16 controls (47%), without any statistically significant differences between the two groups (chi- square test, $p=0.1$). In the group with cancers, the genetic analysis revealed the presence of Ile/Ile common homozygous variant of GSTP1 polymorphism at position 105 in 13 patients (38.2%), Ile/Val heterozygous variant in 15 patients (44.1%) and Val/Val homozygous variant in 2 patients (5.8%). In the control group, we found the Ile/Ile homozygous

variant of GSTP1 at position 105 in 19 subjects (55.8%), Ile/Val heterozygous variant in 15 subjects (44.1%) and Val/Val homozygous variant in none of the subjects. Alleles were in Hardy-Weinberg equilibrium (chi-square test = 0, p=0.9). There were no statistically significant differences in allele frequency between the case group and the controls (chi-square test, p=0.4). For GSTM1, there were no statistically significant differences in null allele frequencies between different cancer locations in the urinary system (chi-square test, $p>0.05$). For GSTT1, there were no statistically significant differences in null allele frequency between the various cancer locations in the urinary system (chi-square test, $p>0.05$). GSTP1 genotype frequency (common and variant in position 105) did not differ between various cancer locations in the urinary system (chi-square test, $p>0.05$). GSTT1 null genotype was found in 9 patients (26.4%) but in no witness, with statistically significant differences between the groups (chi-square test, p=0.002). At least one null genotype (GSTM1 or GSTT1) was found in 28 patients (82.35%) in the case group, and in 16 subjects (47%) in the witness group, revealing a statistically significant difference (chi-square test, p=0.005). In the case group, 4 patients (11.7%) carried a null genotype for both GSTM1 and GSTT1, but the differences were not statistically significant (chi-square test, p=0.1).

Another group consisted of 23 patients with a cancer in the urinary system associated with a second cancer of a different location than the urinary system, together with 23 controls close in age (±2 years) to cancer patients. The average baseline age of patients with multiple malignancies was 65.22 ± 8.2 years (ranging between 50 and 78 years). The average age for the witness group was 64.61 ± 10.08 years (ranging between 50 and 82 years). The average age at the onset of the first cancer was 58.52 ± 10.1 years (ranging between 41 and 78 years). Patient and witness age had a normal distribution (Kolomorov-Smirnov test). There were no statistically significant differences between the average age of the patients at study enrollment and that of the controls (t test for independent variables, p=0.8). The study included 14 women (30%) and 32 men (69.5%). There were 12 smokers among the patients (52.1%) and 13 smokers among the controls (56.2%). The percentage of smokers was not statistically significantly higher in the case group compared to the control group (chi-square test, p=1). GSTM1 null genotype was found in 12 patients (52.1%) and in 1 control (4.3%), revealing statistically significant differences (chi-square test, p=0.001). GSTT1 null genotype was found in 8 patients (34.7%) and in 1 control (4.3%), revealing statistically significant differences (chi-square test, p=0.02). The cancer group noted the presence of the common homozygous variant Ile/Ile of the GSTP1 polymorphism at position 105 in 9 patients (50 %), of the heterozygous variant Ile/Val in 7 patients (38.8%) and of the homozygous variant Val/Val in 2 patients (11.1%). In the control group, the homozygous variant Ile/Ile of the GSTP1 polymorphism at position 105 was found in 11 subjects (47.8%), the heterozygous variant Ile/Val in 12 subjects (52.2%) and the Val/Val genotype was not found in any of the subjects. Alleles were in Hardy-Weinberg equilibrium (chi-square test = 0.9, p=0.4). There were no statistically significant differences in genotype frequency between the patient group and the controls (chi-square test, p=0.2). Seventeen patients (73.9%) in the case group and 2 subjects (8.6%) in the control group carried at least one null genotype (GSTM1 and GSTT1), the percentage difference being statistically significant (chi-square test, p=0.005). In the case group, 3 patients (13%) carried both null genotypes (GSTM1 and GSTT1). The percentage difference was not statistically significant (chi-square test, p=0.2).

Discussion

Globally, prostate cancer is currently second in men (899,000 new cases, representing 13.6% of the total) and fifth overall. In 2008, it was the sixth cancer leading cause of death in men (6.1% of the total). In 2008, Romania (5) had an incidence rate of 19.9 per 100,000 inhabitants (3,620 cases) and a mortality rate of 8.9 (1,757 deaths). U.S. estimates for 2012 rank prostate cancer first (29% of all cancers), bladder cancer fourth (7% of all cancers) and kidney cancer sixth (5% of all cancers) in men, and in women kidney cancer alone represents 3% of all cancers diagnosed, being ranked eighth (6). Mortality rates for cancers of the urinary system rank prostate cancer second (9%) after lung cancer, bladder cancer eighth (3% of all cancers) and kidney cancer tenth (3% of all cancer deaths) in men. Bladder cancer has the following risk factors: smoking (accounting for 50-65% of cases occurring in men and 20-30% of those occurring in women), occupational exposure to aromatic amines, and to a lesser extent cyclophosphamide-based chemotherapy, analgesics containing phenacetin, ionizing radiation, and high levels of arsenic in drinking water (7). Risk factors for kidney cancer include smoking, obesity and hypertension (8-10) (considering that around 20-40% of kidney cancers in men and 10-20% of those in women are due to smoking, 20% to overweight), dialysis (11) (3-26 times increased risk compared to the general population), some syndromes (von Hippel-Lindau). The most common are individuals with two tumors among patients with multiple primitive cancers, those with three distinct tumors only being diagnosed in about 0.5% of patients, and patients with four or five primitive malignancies account for less than 0.1% of the population (12-15). Among our patients, with at least one cancer of the urinary system, five (0.2%) developed three distinct primitive malignancies (16).

From the patients with bladder cancer in our study, 5.18% have developed a second malignant primitive tumor. From those with prostate cancer, 4.11% have also developed other malignant tumors. From the patients with kidney cancer, 10.57% have developed other cancers, either before or after kidney cancer. Some studies have demonstrated an association of up to 70% of prostate cancer in patients with bladder cancer and of 3.4 % of bladder cancers in patients with prostate cancer (17). The literature reveals a low overall risk of developing other primitive malignancies, secondary to prostate cancer (18-21). In our study, 8 patients developed bladder cancer after one prostate cancer, after an average time interval

of three years. It is considered that radiotherapy is one of the factors that may contribute to the development of bladder cancer (22, 23), or other cancers (24-27), after prostate cancer. The number of cases in our study is too small to make relevant assumptions on the role of radiotherapy in further cancer development, but it is likely that such a study conducted at national level might offer a clearer picture. The average latency period between exposure to radiation and the development of bladder cancer is 30 years for low doses and 16.5 years for higher doses (28), although recently it has been reported to be shortened to 5 years (29). In our cases, we could not establish a causal relationship between the therapy applied for prostate cancer and the occurrence of bladder cancer, especially since the average time elapsed between the occurrence of the two primitive malignancies was low (3 years). The patients in our study revealed a higher frequency of either synchronous (in 4 patients renal cell cancer was synchronous with bladder or prostate cancer, in 3 patients bladder cancer was synchronous with prostate cancer) or metachronous association between prostate and bladder cancer, as well as between kidney and bladder or prostate cancers. Urologic cancer associations with other primitive malignant tumors were fewer (most commonly involving the colon, then the breast, hematologic cancers such as lymphomas or leukemia, melanoma and non melanoma skin cancers, larynx). The result of the assessment of all patients with urologic cancers shows that the most common cancer in this area has been bladder cancer, followed by prostate and then by kidney cancers, the most frequently cited in the literature being prostate cancers (30).

GSTs are phase 2 enzymes responsible for catalyzing the biotransformation of a wide variety of electrophilic compounds, thus playing a central role in the detoxification of active metabolites of procarcinogens produced by phase 1 reactions (31). So far, the studies conducted have shown an increase in the GSTM1 null genotype from 47% to 58%, as well as in the GSTT1 null genotype from 13% to 25% in the white European population (32-34), and even up to 80% in Asians. The heterozygous genotype Ile105Val of GSTP1 showed an increase from 38% to 45.7% and the homozygous genotype Val105Val from 7% to 13% (35). The frequency of GSTP1 genotype at position 105 in the Caucasian population was found to be 51.5% Ile/Ile, 39.4% Ile/Val and 9.1% Val/Val (36). It is said that GSTM1 in particular, as well as GSTT1, may be involved in the etiology of cancer in general, and not just one in particular (37). In our study, we found it is a risk factor for mulktiple colorectal cancers (38). Some studies have shown an increased risk for prostate cancer (39-44) associated with polymorphisms in GST genes, although some recent meta-analysis have reported that GSTM1, GSTT1 and GSTP1 polymorphisms are not major determinants of an increased susceptibility to prostate cancer (45). But the risk can become significant in combination with other factors (smoking). Recent studies have observed an increased risk for bladder cancer in GSTM1 null genotype (46-48), the risk being double in association with GSTT1 null genotype, especially in smokers (49). Even if GSTM1 null genotype causes an increased risk of solid tumors (50-53), we have not found an increased risk in patients with urologic cancers. We found GSTT1 null genotype in association with an increased susceptibility to urologic cancers, like other authors (54-58). The variant genotype of GSTP1 is associated with a risk for developing bladder cancer (59, 60). Our patients with multiple urologic cancers revealed no statistically significant differences for GSTP1 polymorphism, but they had a rate of about 50% of at least one modified allele at position 105.

The 23 patients in our second group had other types of cancer, most often colorectal cancer, as well as others (lymphoma, melanoma, breast, gastric, laryngeal, skin cancer), these types of cancers being associated with GSTM1 and GSTT1 polymorphisms (61-64). Although our patients with multiple urologic cancers, compared with controls, had a higher proportion of the GSTM1 null genotype (67.6% versus 47%), this difference was not statistically significant ($p=0.1$), we found that GSTT1 for these patients was significantly more frequent as null genotype (26.4%, $p=0.002$).

Statistically significant results have also been obtained in patients who had at least one GSTM1 or GSTT1 null genotype (82.35% vs. 47% for controls, $p=0.005$). Therefore, we can say that for patients with multiple urinary tumors, GSTT1 and partly GSTM1 may be risk factors. In patients with urologic cancers (kidney, prostate, bladder) associated with other cancers, both GSTM1 null genotype (52.1% versus 4.3%, $p=0.001$) and GSTT1 null genotype (34.7% versus 4.3%, $p=0.002$) were more frequent. The presence of any of the GSTM1 or GSTT1 null genotypes represented a risk factor (73.9% vs. 8.6%, $p=0.005$) in patients with multiple urologic cancers. GSTM1 null genotype may have contributed especially to the development of the other cancers, not so much to that of urologic cancers. We could not assess the contribution of each type of cancer found in the urinary system depending on the association with altered GSTM1, GSTT1 and GSTP1 genotypes. GSTM1 null genotype was found in 18 patients who had at least one bladder cancer, in 20 patients who had at least one prostate cancer, and in 20 patients who suffered from at least one kidney cancer. GSTT1 null genotype was present in 8 patients who developed at least one bladder cancer, in 8 patients who had prostate cancer, and in 10 patients with one primitive malignant tumor in the kidney. Heterozygous GSTP1 Ile105Val genotype was found in 13 patients with bladder cancer, in 9 patients with prostate cancer, and in 13 patients with kidney cancer. Homozygous GSTP1 Val105Val genotype was found in one patient with bladder cancer, in one with prostate adenocarcinoma, and in two patients with one of the cancers found in the kidney.

Conclusions

GSTT1 null genotype is a risk factor for patients with multiple malignant primitive urologic tumors (bladder, prostate and kidney). GSTM1 and GSTT1 null genotypes are more frequent in patients with multiple urologic malignancies. GSTM1 and GSTT1 null genotypes are risk factors in patients with different types of cancer, of which at least

one is in the urinary system. It is possible that the results linked to the GSTM1 null genotype may be due to other cancers, not to the one found in the urinary system (in the bladder, kidney or prostate). GSTM1 null genotype and GSTP1 variant genotype were not risk factors in patients with multiple urologic cancers.

Acknowledgements

We would like to thank the clinicians and other hospital staff who contributed to the blood samples and data collection for this study. We thank all the patients and individuals for their participation in this study.

Conflict of interest

We declare that there is no conflict of interest for this manuscript. The work described is original and has not been published previously, and not under consideration for publication elsewhere.

References

1. Chirila DN, Turdeanu NA, Constantea NA, Coman I, Pop T, Popp RA, et al. Multiple malignant tumors. Chirurgia (Bucur). 2013;108(4):498-502.
2. Bajpai P, Tripathi A. K., Agrawal D. Increased frequencies of glutathione-S-transferase (GSTM1 and GSTT1) null genotypes in Indian patients with chronic myeloid leukemia. Leukemia Research. 2007;31(10):1359-1363.
3. Harries LW, Stubbins MJ, Forman D, Howard GC, Wolf CR. Identification of genetic polymorphisms at the glutathione S-transferase Pi locus and association with susceptibility to bladder, testicular and prostate cancer. Carcinogenesis. 1997;18:641–4.
4. Hohaus S, Di Ruscio A, Di Febo A, Massini G, D'Alo' F, Guidi F, et al. Glutathione S-transferase P1 Genotype and Prognosis in Hodgkin's Lymphoma. Clin Cancer Res. 2005;11:2175–2179.
5. Ferlay J, Shin HR, Bray F, Forman D, Mathers C and Parkin DM.GLOBOCAN 2008 v2.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 10 (Internet).Lyon, France: International Agency for Research on Cancer; 2010. Available from: http://globocan.iarc.fr, accessed on 24/December/2011.
6. Cancer Facts and Figures 2012. Available from http://www.cancer.org/acs/groups/content/@epidemiology-surveilance/documents/document/acspc-031941.pdf. accessed on 15/November/2012.
7. Taylor Wilson R, Silverman DT, Fraumeni JF Jr, Curtis RE. New Malignancies Following Cancer of the Urinary Tract. In Curtis RE, Freedman DM, Ron E, Ries LAG, Hacker DG, Edwards BK, Tucker MA, Fraumeni JF Jr. (eds). New Malignancies Among Cancer Survivors: SEER Cancer Registries, 1973-2000. National Cancer Institute, NIH Publ. No. 05-5302. Bethesda, MD, 2006.
8. McLaughlin JK, Lindblad P, Mellemgaard A, McCredie M, Mandel JS, Schlehofer B, et al. International renal-cell cancer study. I. Tobacco use. Int J Cancer. 1995;60(2):194-8.
9. Lipworth L, Tarone RE, Lund L, McLaughlin JK. Epidemiologic characteristics and risk factors for renal cell cancer. Clin Epidemiol. 2009;1:33-43.
10. Chow WH, Dong LM, Devesa SS. Epidemiology and risk factors for kidney cancer. Nat Rev Urol. 2010;7(5):245-57.
11. Stewart JH, Buccianti G, Agodoa L, Gellert R, McCredie MR, Lowenfels AB, et al. Cancers of the kidney and urinary tract in patients on dialysis for end-stage renal disease: analysis of data from the United States, Europe, and Australia and New Zealand. J Am Soc Nephrol. 2003;14(1):197-207.
12. Mussari S, Amichetti M, Tomio L. Quadruple cancer in a single patient: a report of four cases. Eur J Surg Oncol. 2000; 26: 614-616.
13. Yhim HY, Kim HS, Lee NR, Kwak JY, Yim CY, Park HS, et all. Quadruple primary malignancies of liver, bladder, lung and stomach in one patient.Tumori. 2010;96(5):787-91.
14. Nishitani H, Hosomi M, Akashi H, Hori K, Kitagawa H, Matsumoto M. A case of quintuple primary malignant tumors. Nippon Naika Gakkai Zasshi. 1995; 84: 122-123.
15. Martin-Granizo R, Naval L, Castro P, Goizueta C. Munoz M. Quintuple cancers: report of a case with triple cancers in the head and neck. J Craniomaxillofac Surg. 1997; 25: 153-157.
16. Chirilă DN, Popp R, Bălăcescu O, Coman I, Constantea NA, Turdeanu NA, et al. The glutahione S-transferases (GSTM1, GSTT1 and GSTP1) polymorphisms in patients with three malignant primary tumors. Ann Rom Soc Cell Biol. 2012; XVII(2):111-120.
17. Kinoshita Y, Singh A, Rovito PM Jr, Wang CY, Haas GP. Double primary cancers of the prostate and bladder: a literature review. Clin Prostate Cancer. 2004;3(2):83-6.
18. McMaster ML, Feuer E J, Tucker MA. Chapter 10 New Malignancies Following Cancer of the Male Genital Tract. In NEW MALIGNANCIES AMONG CANCER SURVIVORS: SEER CANCER REGISTRIES, 1973–2000. 2011:257-284 from http://seer.cancer.gov/publications/mpmono/Ch10_MaleGenital.pdf, accessed on 23/July/2012
19. Osterlind A, Rørth M, Prener A. Second cancer following cancer of the male genital system in Denmark, 1943-80. Natl Cancer Inst Monogr. 1985;68:341-7.
20. Kleinerman RA, Liebermann JV, Li FP. Second cancer following cancer of the male genital system in Connecticut, 1935-82. Natl Cancer Inst Monogr. 1985;68:139-47.
21. Nielsen SF, Nordestgaard BG, Bojesen SE. Associations between first and second primary cancers: a population-based study. CMAJ. 2012;184(1): E57–E69.
22. Neugut AI, Ahsan H, Robinson E, Ennis RD. Bladder carcinoma and other second malignancies after radiotherapy for prostate carcinoma. Cancer. 1997;79(8):1600-4.
23. Shirodkar SP, Kishore TA, Soloway MS. The risk and prophylactic management of bladder cancer after various forms of radiotherapy. Curr Opin Urol. 2009;19(5):500-3.
24. Brenner DJ, Curtis RE, Hall EJ, Ron E. Second malignancies in prostate carcinoma patients after radiotherapy compared with surgery. Cancer. 2000;88(2):398-406.
25. Nieder AM, Porter MP, Soloway MS. Radiation therapy for prostate cancer increases subsequent risk of bladder and rectal cancer: a population based cohort study. J Urol. 2008;180(5):2005-9; discussion 2009-10.
26. Singh AK, Mashtare TL, McCloskey SA, Seixas-Mikelus SA, Kim HL, May KS. Increasing age and treatment modality are predictors for subsequent diagnosis of bladder cancer following prostate cancer diagnosis. Int J Radiat Oncol Biol Phys. 2010;78(4):1086-94.
27. Sountoulides P, Koletsas N, Kikidakis D, Paschalidis K,

Sofikitis N. Secondary malignancies following radiotherapy for prostate cancer. Ther Adv Urol. 2010;2(3):119-25.
28. Quilty PM, Kerr GR. Bladder cancer following low or high dose pelvic irradiation. Clin Radiol. 1987;38(6):583-5.
29. Suit H, Goldberg S, Niemierko A, Ancukiewicz M, Hall E, Goitein M, et al. Secondary carcinogenesis in patients treated with radiation: a review of data on radiation-induced cancers in human, non-human primate, canine and rodent subjects. Radiat Res. 2007;167(1):12-42.
30. Tiszlavicz L, Szalay I. Multiple urologic tumors in autopsy material. Orv Hetil. 1993;134(11):577-81.
31. Nebert DW, Vasiliou .V Analysis of the glutathione S-transferase (GST) gene family. Hum Genomics. 2004;1(6):460-4.
32. Garte S, Gaspari L, Alexandrie AK, Ambrosone C, Autrup H, Autrup JL, et al. Metabolic gene polymorphism frequencies in control populations. Cancer Epidemiol Biomarkers Prev. 2001;10(12):1239-48.
33. Jang TL, Yossepowitch O, Bianco FJ, Jr, Scardino PT. Low risk prostate cancer in men under age 65: the case for definitive treatment. Urol Oncol. 2007;25:510–514.
34. Hankey B, Feuer EJ, Clegg LX, Hayes RB, Legler JM, Prorok PC, et al. Cancer surveillance series: interpreting trends in prostate cancer-part I: Evidence of the effects of screening in recent prostate cancer incidence, mortality, and survival rates. J Natl Cancer Inst. 1999;91:1017–1024.
35. Watson MA, Stewart RK, Smith GB, Massey TE, Bell DA. Human glutathione S-transferase P1 polymorphisms: relationship to lung tissue enzyme activity and population frequency distribution. Carcinogenesis. 1998;19(2):275-80.
36. Ryberg D, Skaug V, Hewer A, Phillips DH, Harries LW, Wolf CR, et al. Genotypes of glutathione transferase M1 and P1 and their significance for lung DNA adduct levels and cancer risk. Carcinogenesis. 1997;18(7):1285-9.
37. Rebbeck TR. Molecular epidemiology of the human glutathione S-transferase genotypes GSTM1 and GSTT1 in cancer susceptibility. Cancer Epidemiol Biomarkers Prev. 1997;6(9):733-43.
38. Chirila DN, Popp RA, Balacescu O, Turdeanu NA, Constantea NA, Pop TR, et al. GST gene variants in synchronous colorectal cancers and synchronous association of colorectal cancers with other cancers. Chirurgia (Bucur). 2013;108(3):365-71.
39. Medeiros R, Vasconcelos A, Costa S, Pinto D, Ferreira P, Lobo F, et al. Metabolic susceptibility genes and prostate cancer risk in a southern European population: the role of glutathione S-transferases GSTM1, GSTM3, and GSTT1 genetic polymorphisms. Prostate. 2004;58(4):414-20.
40. Ntais C, Polycarpou A, Ioannidis JP. Association of GSTM1, GSTT1, and GSTP1 gene polymorphisms with the risk of prostate cancer: a meta-analysis. Cancer Epidemiol Biomarkers Prev. 2005;14(1):176-81.
41. Agalliu I, Langeberg WJ, Lampe JW, Salinas CA, Stanford JL. Glutathione S-transferase M1, T1, and P1 polymorphisms and prostate cancer risk in middle-aged men. Prostate. 2006;66(2):146-56.
42. Lavender NA, Benford ML, VanCleave TT, Brock GN, Kittles RA, Moore JH, et al. BMC Examination of polymorphic glutathione S-transferase (GST) genes, tobacco smoking and prostate cancer risk among men of African descent: a case-control study. Cancer. 2009;9:397.
43. Qadri Q, Sameer AS, Shah ZA, Hamid A, Alam S, Manzoor S, et al. Genetic polymorphism of the glutathione-S-transferase P1 gene (GSTP1) and susceptibility to prostate cancer in the Kashmiri population. Genet Mol Res. 2011;10(4):3038-45.
44. Gong M, Dong W, Shi Z, Xu Y, Ni W, An R. Genetic Polymorphisms of GSTM1, GSTT1, and GSTP1 with Prostate Cancer Risk: A Meta-Analysis of 57 Studies. PLoS One. 2012;7(11):e50587. doi: 10.1371/journal.pone.0050587. Epub 2012 Nov 26.
45. Katoh T, Yamano Y, Tsuji M, Watanabe M. Genetic polymorphisms of human cytosol glutathione S-transferases and prostate cancer. Pharmacogenomics. 2008;9(1):93-104.
46. Bell DA, Taylor JA, Paulson DF, Robertson CN, Mohler JL, Lucier GW. Genetic risk and carcinogen exposure: a common inherited defect of the carcinogen-metabolism gene glutathione S-transferase M1 (GSTM1) that increases susceptibility to bladder cancer. J Natl Cancer Inst. 1993; 85:1159-64.
47. Georgiou I, Filiadis IF, Alamanos Y, Bouba I, Giannakopoulos X, Lolis D. Glutathione S-transferase null genotypes in transitional cell bladder cancer: a case-control study. Eur Urol. 2000;37(6):660-4.
48. Engel LS, Taioli E, Pfeiffer R, Garcia-Closas M, Marcus PM, Lan Q, et al. Pooled analysis and meta-analysis of glutathione S-transferase M1 and bladder cancer: a HuGE review. Am J Epidemiol. 2002;156(2):95-109.
49. Salinas-Sánchez AS, Sánchez-Sánchez F, Donate-Moreno MJ, Rubio-del-Campo A, Gimenez-Bachs JM, Lorenzo-Romero JG, et al. Polymorphic deletions of the GSTT1 and GSTM1 genes and susceptibility to bladder cancer. BJU Int. 2011;107(11):1825-32.
50. Harada S, Misawa S, Nakamura T, Tanaka N, Ueno E, Nozoe M. Detection of GST1 gene deletion by the polymerase chain reaction and its possible correlation with stomach cancer in Japanese. Hum Gene. 1992; 90(1-2):62-4.
51. Nakajima T, Elovaara E, Anttila S, Hirvonen A, Camus AM, Hayes JD, et al. Expression and polymorphism of glutathione S-transferase in human lungs: risk factors in smoking-related lung cancer. Carcinogenesis. 1995; 16(4):707-11.
52. Agalliu I, Lin DW, Salinas CA, Feng Z, Stanford JL. Polymorphisms in the glutathione S-transferase M1, T1, and P1 genes and prostate cancer prognosis. Prostate. 2006;66(14):1535-41.
53. Wei B, Xu Z, Zhou Y, Ruan J, Cheng H, Xi B, et al. Association of GSTM1 Null Allele with Prostate Cancer Risk: Evidence from 36 Case-Control Studies. PLoS One. 2012;7(10):e46982.
54. Komiya Y, Tsukino H, Nakao H, Kuroda Y, Imai H, Katoh T. Human glutathione S-transferase A1, T1, M1, and P1 polymorphisms and susceptibility to prostate cancer in the Japanese population. J Cancer Res Clin Oncol. 2005;131(4):238-42.
55. Katoh T, Inatomi H, Kim H, Yang M, Matsumoto T, Kawamoto T. Effects of glutathione S-transferase (GST) M1 and GSTT1 genotypes on urothelial cancer risk. Cancer Lett. 1998; 132(1-2):147-52.
56. Törüner GA, Akyerli C, Uçar A, Aki T, Atsu N, Ozen H, et al. Polymorphisms of glutathione S-transferase genes (GSTM1, GSTP1 and GSTT1) and bladder cancer susceptibility in the Turkish population. Arch Toxicol. 2001;75(8):459-64.
57. Ahmad ST, Arjumand W, Seth A, Kumar Saini A, Sultana S. Impact of glutathione transferase M1, T1, and P1 gene polymorphisms in the genetic susceptibility of North Indian population to renal cell carcinoma. DNA Cell Biol. 2012;31(4):636-43.
58. Ha YS, Yan C, Park C, Yun SJ, Moon SK, Choi YH, et al.

GSTT1: a marker of the aggressiveness of bladder cancer. Urol Int. 2011;86(1):41-6.
59. Kellen E, Hemelt M, Broberg K, Golka K, Kristensen VN, Hung RJ, et al. Pooled analysis and meta-analysis of the glutathione S-transferase P1 Ile 105Val polymorphism and bladder cancer: a HuGE-GSEC review. Am J Epidemiol. 2007;165(11):1221-30.
60. Safarinejad MR, Safarinejad S, Shafiei N, Safarinejad S. Association of genetic polymorphism of glutathione S-transferase (GSTM1, GSTT1, GSTP1) with bladder cancer susceptibility. Urol Oncol. 2011 Dec 9. (Epub ahead of print)
61. Singh M, Shah PP, Singh AP, Ruwali M, Mathur N, Pant MC, et al. Association of genetic polymorphisms in glutathione S-transferases and susceptibility to head and neck cancer. Mutat Res. 2008;638(1-2):184-94.
62. Chen B, Cao L, Zhou Y, Yang P, Wan HW, Jia GQ, et al. Glutathione S-transferase T1 (GSTT1) gene polymorphism and gastric cancer susceptibility: a meta-analysis of epidemiologic studies. Dig Dis Sci. 2010;55(7):1831-8.
63. Zhang Y, Ni Y, Zhang H, Pan Y, Ma J, Wang L. Association between GSTM1 and GSTT1 Allelic Variants and Head and Neck Squamous Cell Carcinoma. PLoS One. 2012;7(10):e47579.
64. Cadoni G, Boccia S, Petrelli L, Di Giannantonio P, Arzani D, Giorgio A, et al. A review of genetic epidemiology of head and neck cancer related to polymorphisms in metabolic genes, cell cycle control and alcohol metabolism. Acta Otorhinolaryngol Ital. 2012;32(1):1-11.