Level of selenoprotein transcripts in peripheral leukocytes of patients with bladder cancer and healthy individuals

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Abstract

Background: Low concentrations of selenium (Se) in humans have been associated with risk of cancer. Selenoprotein mRNAs can potentially be regulated by Se status.

Methods: Se status, *GPx1* Pro198Leu and *Sep15* 1125G/A genetic polymorphism and human *(h)GPx1*, *hGPx3*, *hSep15* and *hSeP1* transcript levels in peripheral leukocytes of 33 males with bladder cancer and 47 healthy male controls were analysed.

Results: All the subjects expressed detectable selenoprotein mRNA concentrations in leukocytes. Significantly lower expression of *hGPx1*, *hGPx3, hSep15* and *hSeP1* in leukocytes of bladder cancer patients compared to controls was observed. *hGPx1*, *hGPx3* and *hSep15* expression was significantly lower in non-smokers in the control group compared with smokers in the control group. A positive relationship between expression of all studied genes was also observed in non-smoking controls. Expression of *hGPx3* and *hSep15* gradually increased with tumour grade in patients with cancer. We did not find any association between selenoprotein mRNA levels, Se status and selenoprotein genetic polymorphism.

Conclusions: This study showed significant down-regulation of *hGPx1*, *hGPx3*, *hSep15* and *hSeP1* mRNA levels in leukocytes of patients with bladder cancer compared to controls. Selenoprotein transcript levels in circulating leukocytes of patients with bladder cancer and controls revealed no potential impact of Se status on selenoprotein expression.

Clin Chem Lab Med 2009;47:1125–32.

Keywords: bladder cancer; genetic polymorphism; leukocytes; real-time PCR; selenoproteins.

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Introduction

Bladder cancer is the seventh most common cancer in men in the world, and fourth most common in Poland. The incidence is higher in Polish males than in males from Central and Eastern European countries, but similar to that observed in Western and Southern Europe and the US (1). Well known risk factors for bladder cancer include tobacco smoking, occupational exposure to aromatic amines from rubber or paint and chronic infections with *schistosoma haematobium*. Some additional risk factors, such as diet and nutritional habits have also been considered (2).

Selenium (Se) is a dietary trace element that is essential for human health (3). Low Se concentrations measured in blood, toenails and hair have been associated with lung, prostate and colorectal cancer (4), although epidemiological studies on Se status and bladder cancer risk showed contradictory results (5). One of the proposed anticancer mechanisms of Se suggests that there are antioxidative properties of selenoproteins. Se, genetically encoded as the 21st aminoacid selenocysteine, is reportedly present at the active sites of 25 selenoproteins in humans, including five Se-dependent glutathione peroxidases: cytosolic glutathione peroxidase (GPx1), extracellular plasma glutathione peroxidase (GPx3), gastrointestinal GPx2, phospholipid hydroperoxide-reducing GPx4, oilfactory epithelial GPx6, and also selenoprotein P (SeP), 15 kDa selenoprotein (Sep15), three iodothyronine deiodinases, three thioredoxin (TrR) reductases and additional selenoproteins (6).

Differences in the expression of selenoprotein mRNAs can reflect dietary Se supply. Both *GPx1* and *GPx3* transcript levels rapidly decrease in cases of Se deficiency, whereas *GPx2* mRNA and *GPx4* mRNA levels are fairly resistant to deficiency (7, 8). This hierarchy of selenoproteins with respect to Se status may be important in the formation of different tumours, especially since selenoproteins show tissue-specific expression profiles (8). In vitro and animal studies suggest that low levels of selenoprotein transcripts in response to low Se concentrations have been associated with loss of mRNA stability, and degradation during non-sense-mediated decay (NMD) process (7, 8). Of the 25 known human selenoprotein mRNAs, 14 are thought to be sensitive to NMD (9). Single nucleotide polymorphisms, potentially modifying transcription processes, have also been observed in genes encoding for selenoproteins (10).

Special emphasis has recently been placed on the application of circulating white blood cell (WBC) transcriptome patterns. Transcriptomic studies clearly

show that various cluster genes can be significantly expressed in WBCs (11). This allows genomic fingerprints to be easily obtained to explore the different responses to nutritional status and environmental exposure. Gene expression patterns may be useful for defining biological processes associated with human health and disease. Evenson et al. (12) found that mRNA concentrations of rat selenoproteins in whole blood were comparable to concentrations detected in the major organs. Decreased rat *GPx1* mRNA concentrations in rat blood and liver under conditions of dietary deficiency of Se indicated that WBC mRNA concentrations could be a biomarker of Se exposure in rats. Lampe et al. (13) showed that active exposure to tobacco smoke is associated with a biological relevant mRNA expression signature. Therefore, we investigated human *(h)GPx1, hGPx3, hSep15*, and *hSeP1* transcript concentrations in circulating leukocytes to determine whether gene expression in healthy individuals differs from that in patients with bladder cancer who present with different smoking habits. Also, we wished to determine if there is an impact of Se status and *GPx1* Pro198Leu, *Sep15* 1125G/A genetic polymorphism on expression of selenoprotein mRNA.

Materials and methods

Study population

Male patients with bladder cancer and healthy male controls were recruited from the Department of Urology, Medical University, Lodz, and from the Nofer Institute of Occupational Medicine, Lodz, respectively. All patients were not undergoing intravenous and/or intrabladder chemotherapy administration and with transurethral resection. Patients presented with transitional cell carcinoma (TCC) of the urinary bladder. Cases (n=33) and controls (n=47) were selected with respect to smoking habits in order to provide similar numbers of smokers and non-smokers. The non-smokers included those who had never smoked and those who previously smoked, but quit smoking ≥5 years prior to entering

the study. The study participants completed a questionnaire that provided information on demographic characteristics including age, body mass index (BMI) and smoking history. The characteristics of the study population are presented in Table 1. The Regional Ethics Committee for Scientific Research approved the study protocol, and a written informed consent was obtained from all participants.

Se status

Blood samples were collected into Venoject® heparinized (lithium heparin) test tubes, free from trace elements, for determination of Se status. Blood was also collected into EDTA tubes for isolation of RNA and DNA. Plasma Se concentrations were measured according to Neve et al. (14) using graphite furnace atomic absorption spectrometry (Solaar 989 QZ apparatus, Unicam, Thermo Scientific, Warsaw, Poland). The method was validated using reference material consisting of lyophilized human reference serum samples of Seronorm™ (Nycomed Pharma AS, Oslo, Norway). Red blood cell (RBC) and plasma GPx activities were measured spectrophotometrically according to the method of Paglia and Valentine (15) with modification by Hopkins and Tudhope (16).

RNA and DNA isolation and cDNA synthesis

Total RNA and genomic DNA were isolated from whole blood using the QIAamp RNA Blood Mini Kit and QIAamp DNA Blood Mini Kit (Qiagen, Syngen Biotech, Wroclaw, Poland), respectively, according to the manufacturer's instructions. The concentration and purity of isolated RNA and DNA were determined spectrophotometrically. cDNA was synthesized on 250 ng RNA with Omniscript RT Kit (Qiagen, Syngen Biotech, Poland), oligo dT_{15-18} primer and RNase-free DNase I (Promega, Symbios, Gdansk, Poland).

Selenoprotein mRNAs expression

Expression of the *hGPx1*, *hGPx3*, *hSep15* and *hSeP1* genes was quantified with the iQ5 real-time PCR detection system (Bio-Rad, Warsaw, Poland) using glyceraldehyde 3-phosphate dehydrogenase (*hGAPDH*) as the endogenous control. This allowed for stable expression levels of RNA isolated from peripheral blood leukocytes. Forward and reverse primers for target genes and TaqMan® probes were designed

Table 1 Demographics of the study population.

	Controls (n=47)	Cancer (n=33)	p-Value
Age, years	49.1±17.4	67.6±9.7	<0.0001
Smoking habit			
Yes	13 (27.7%)	13 (39.4%)	0.335
No	34 (72.3%)[a]	20 (60.6%)[b]	
BMI[c], kg/m²	25.1±4.0	26.7±3.3	0.120
Se, ng/mL plasma	54.3±14.6	50.5±23.0	0.282
GPx1, U/L plasma	22.9±3.7	22.0±7.2	0.434
GPx3, U/g Hb	0.200±0.040	0.167±0.036	0.0008
GPx1			
Pro198Pro	27 (58.7%)[d]	13 (39.4%)	0.058
Pro198Leu	18 (39.1%)	15 (45.5%)	
Leu198Leu	1 (2.2%)	5 (15.1%)	
Sep15			
1125G/G	20 (43.5%)	21 (63.6%)	0.141
1125G/A	23 (50.0%)	9 (27.3%)	
1125A/A	3 (6.5%)	3 (9.1%)	

[a]The non-smoking group includes 2 ex-smokers, who quit smoking ≥5 years before the investigation; [b]the non-smoking group includes 15 ex-smokers, who quit smoking ≥5 years before the diagnosis of cancer, smoking history of 1 individual is missing; [c]BMI data on 3 controls are missing; [d]*GPx1* genotyping data for 1 control is missing. Hb, hemoglobin.

with Beacon Designer 7.0 (PREMIER Biosoft Int, Bio-Rad, Poland) according to the GenBank® genetic sequence database. The amplicon sequence complied with exon-exon boundaries, whenever possible (Table 2). All samples were amplified in triplicate. Samples from the controls and cancer patients were amplified simultaneously on the same plate. Calculated PCR efficiencies were: 99% for *hGPx3*, 97% for *hSeP1* and 95% for *hGPx1*, *hSep15* and *hGAPDH*. mRNA expression data were evaluated by means of two calculation methods. Reference gene-normalized relative quantitation with efficiency correction was calculated using Q-Gene, a Microsoft Excel®-based software application coded in Visual Basic (17). Calibrator-normalized relative expression for estimating up- and down-regulation of gene expression in the patients with bladder cancer was calculated using REST 2005 Software, Version: 1.9.10 (The Gene Quantification website, www.gene-quantification.info).

Genotyping

GPx1 Pro198Leu (rs 1050450) primers and probes were designed with Beacon Designer 7.0 (PREMIER Biosoft Int, Bio-Rad, Poland) according to the GenBank® genetic sequence database. Genetic polymorphism was analysed using Molecular Beacon Real-Time PCR. Oligonucleotide sequences for PCR primers and fluorescent probes were: forward primer 5′–TGACGGTGTGCCCCTACG–3′, and reverse primer 5′–CCGAGACAGCAGCACTGC–3′, probe C 5′–(6-Fam) CGCGATCAGGCACAGCTGGGCCCTTGAGACGATCGCG (Dabcyl)–3′, probe T 5′–(Hex) CGCGATCAGGCACAGCTGAG-CCCTTGAGACGATCGCG (Dabcyl)–3′. *Sep15* 1125G/A polymorphism (rs 5859) was analysed using the PCR-RFLP assay. Oligonucleotide sequences for PCR primers were: forward 5′–GCCTGCTCCTCAGAGTCTC–3′ and reverse 5′–AAACATGAAAGAACAAACCAGAAG–3′. The amplicons were digested with *FspB*I (Fermentas, ABO, Gdansk, Poland) and the digestion products (360 bp, 198 bp, 162 bp) were analysed in 1.5% agarose gel using Molecular Imager Gel Doc and ChemiDoc Systems and Quantity One software (Bio-Rad, Poland).

Statistical analysis

Statistical analyses were performed with GraphPad Prism® Version 5.01 (GraphPad Software, Inc., San Diego, CA, USA; www.graphpad.com). For comparison of the study groups, an unpaired t-test, one-way analysis of variance (for biochemical analyses) and the Mann-Whitney U-test, the Kruskal-Wallis test (for gene expression analyses) and Fisher's exact test were used. Correlations were assessed using Pearson (for biochemical analyses) or Spearman (for gene expression analyses) analysis. For all the tests, a two-sided $p < 0.05$ was used for determining statistical significance.

Results

Smoking habits and BMI were similar in both groups. Thirty-three patients with TCC of the urinary bladder, with different grading (G) and staging (T), were examined. The majority of bladder cancer cases were characterized by low degree of neoplasm (G1) and clinical progression (T1) ($n = 14$ and $n = 24$, respectively). Eight patients with grade G2 and nine patients with grade G3 presented with a high degree of neoplasia. Invasive characteristics of bladder tumours were seen in four patients with stage T2 and three patients with grade T3. Histopathological data for grading two of the patients was missing.

Gene expression

All the subjects expressed measurable selenoprotein mRNA concentrations in blood leukocytes, with large inter-individual variations in gene expression. The highest concentration of gene transcripts in circulating leukocytes was observed for the *hGPx1* and *hSep15* genes. The lowest concentration was attributable to the *hGPx3* and *hSeP1* genes. We observed significant down-regulation of *hGPx1*, *hGPx3, hSep15* and *SeP1* mRNA in leukocytes from patients with bladder cancer when compared to controls. *hSeP*1 and *hGPx3* showed the highest down-regulation in the bladder cancer group compared to controls, with mean factors of 0.480 and 0.546, respectively. *hSep15* showed down-regulation in the bladder cancer group, compared to the control group, by a mean factor of

Table 2   Oligonucleotide primers and TaqMan® probes used for real-time PCR.

Gene	GI number	Primers and probes sequence[a]	Amplicon size, bp
hGPx1	41406083	F–GCATCAGGAGAACGCCAAGAAC R–CGCAGGAAGGCGAAGAGAGG P–CCCGCACCGTTCACCTCGCACTTC	144
hGPx3	89903006	F–GAACTCAGAGATCCTTCCTACCC R–CTGCTCTTTCTCTCCATTGACATC P–CCGACCAGGTGGAGGCTTTGTCCC	106
hSep15	42741647	F–TTTGGGCTACGGTTGTTGTTG R–AGTTGCTAGAAAAGCCTAACTCTC P–TGCCTCCGATGAAAACTCTGCCCC	103
hSepP1	62530390	F–GCCTTCATCACCACCATAAGC R–ATACATCTCTTTCGACAGAGCTTC P–TCAGCATAGGCAGGGTCACCCAGA	121
hGAPDH	83641890	F–GGACCTGACCTGCCGTCTAG R–TGTAGCCCAGGATGGCCCTTG P–CCTCCGACGCCTGCTTCACCACCT	101

[a]F, forward 5′–3′; R, reverse primer 5′–3′; P, TaqMan probe 5′–3′ (6-FAM–TAMRA); h, human; GPx1, cytosolic glutathione peroxidase; GPx3, plasma glutathione peroxidase; Sep15, 15 kDa selenoprotein; SeP1, selenoprotein P; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.
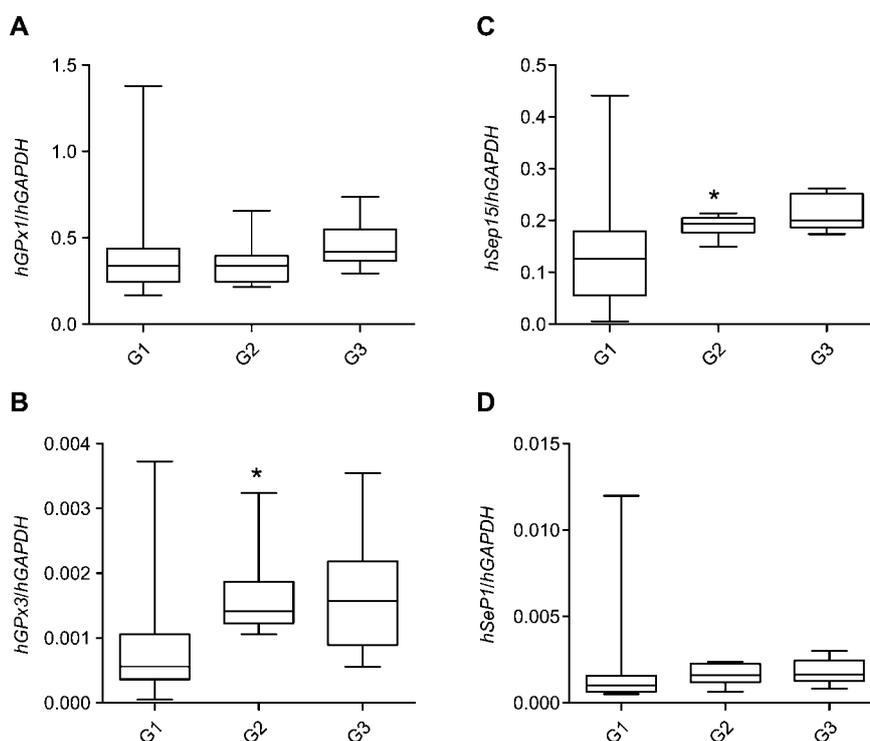
Table 3 Differences in selenoprotein mRNA expression normalised to *hGAPDH*[a] and selenoprotein mRNA relative expression[b] from leukocytes of patients with bladder cancer and controls.

Gene	Controls, n=47	Cancer, n=33	p-Value[a]	Expression ratio (SE)	p-Value[b]
hGPx1					
Median	0.435	0.372	0.190	0.778 (0.360–1.637)	0.067
25% percentile	0.247	0.265			
75% percentile	0.718	0.425			
Mean±SD	0.683±0.705	0.404±0.222			
hGPx3					
Median	0.0018	0.0012	0.019	0.546 (0.198–1.641)	0.002
25% percentile	0.0009	0.0006			
75% percentile	0.0031	0.0019			
Mean±SD	0.0026±0.0028	0.0013±0.0010			
hSep15					
Median	0.205	0.187	0.01	0.592 (0.469–1.082)	<0.0001
25% percentile	0.168	0.132			
75% percentile	0.283	0.210			
Mean±SD	0.279±0.227	0.173±0.078			
hSeP1					
Median	0.0023	0.0013	<0.0001	0.480 (0.246–0.901)	<0.0001
25% percentile	0.0017	0.0009			
75% percentile	0.0038	0.0019			
Mean±SD	0.0045±0.0068	0.0019±0.0020			

[a]Reference gene-normalised relative quantification with efficiency correction; [b]calibrator-normalised relative expression.

0.592 and *hGPx1* by a mean factor of 0.778 (Table 3). We did not observe differences in expression of the four selenoprotein encoding genes in patients with TCC of the bladder and non-invasive (T1) and invasive (T1+T3) characteristics. However, expression of selenoprotein mRNA in leukocytes of patients with TCC gradually increased with tumour grade, showing statistical significance for *hGPx3* (p=0.023) and *hSep15* (p=0.008) (Figure 1B and C). Comparison of G1 and G3 tumour grades showed significant up-regulation of *hGPx3* (2.7-fold; p=0.045) and *hSep15* (2.4-fold; p=0.040) (data not shown). In the control group, significant correlation between expression levels of the selenoproteins (*hGPx1/hGPx3, hGPx1/hSep15, hGPx1/hSeP1, hGPx3/hSep15, hGPx3/hSeP1, hSep15/hSeP1*) were found. However, in patients with cancer, the only significant correlation was found between *hGPx3* and *hSep15* (r=0.350, p=0.05) (Table 4).

Figure 1 Selenoprotein mRNA expression normalised to *hGAPDH* in leukocytes in relation to different grades of bladder cancer (G1, n=14; G2, n=8; G3, n=9).
(A) mRNA *hGPx1*, p=0.124; (B) mRNA *hGPx3*, p=0.023*; (C) mRNA *hSep15*, p=0.008*; (D) mRNA *hSeP1*, p=0.114.

Table 4   Correlation between selenium status and selenoprotein mRNA expression normalised to *hGAPDH* in leukocytes from patients with bladder cancer and controls.

Correlation	Controls, n = 47		Cancer, n = 33	
	r	p-Value	r	p-Value
Se status				
GPx1/Se	0.114	0.444	0.334	0.057
GPx3/Se	0.468	0.0009	0.343	0.051
Selenoprotein mRNAs expression and Se status				
hGPx1/Se	0.180	0.226	0.001	0.995
hGPx3/Se	0.178	0.230	−0.061	0.734
hSep15/Se	0.101	0.501	−0.007	0.710
hSeP1/Se	0.159	0.287	0.069	0.702
hGpx1/GPx1	−0.223	0.132	0.133	0.459
hGPx3/GPx3	0.006	0.967	−0.018	0.922
Selenoprotein mRNAs expression				
hGPx1/*hGPx3*	0.797	<0.0001	0.029	0.871
hGPx1/*hSep15*	0.768	<0.0001	0.316	0.074
hGPx1/*hSeP1*	0.425	0.003	0.186	0.299
hGPx3/*hSep15*	0.791	<0.0001	0.350	0.05
hGPx3/*hSeP1*	0.453	0.001	0.279	0.116
hSep15/*hSeP1*	0.583	<0.0001	0.455	0.008

Se status

Se status in the study participants was assessed using plasma Se concentration, GPx3 activity, and GPx1 activity in RBCs. In both cases and controls, neither Se concentration or GPx1 activity differed significantly. Plasma GPx3 activity was significantly lower (p < 0.0008) in patients with bladder cancer compared with controls (Table 1). Se status did not differ between patients with bladder cancer with different tumour grades (G1, G2 and G3) and characteristics of muscle invasion (T1 and T2 + T3) (data not shown). There was no significant correlation between Se concentrations and GPx activities in both study groups. A significant association between plasma Se concentration and GPx3 activity in plasma (r = 0.468; p < 0.0009) was found in controls (Table 4). An analysis performed to assess the relationship between selenoprotein mRNA concentrations and Se status, indicated by Se concentrations and plasma activity of GPx1 RBC and GPx3, revealed no significant correlation in either of the groups.

Genetic polymorphism

The distribution of *GPx1* Pro198Leu and *Sep15* 1125G/A genotypes in the controls and patients with bladder cancer did not differ significantly (Table 1). We found no effect of genetic polymorphisms of *GPx1* and *Sep15* on Se status or selenoprotein mRNA concentrations (data not shown).

Impact of smoking on Se status and gene expression

To investigate the potential impact of tobacco smoking on Se status and selenoprotein gene expression, we divided both groups into non-smokers and smokers. There were no differences between selenoprotein transcript concentrations in bladder cancer patients, relative to smoking habits. However, in the control group, *hGPx1*, *hGPx3* and *hSep15* expression was significantly lower in smokers compared with non-smokers. *hSep15* showed the highest down-regulation in smokers compared with non-smokers by a mean factor of 0.645. *hGPx3* was down-regulated by a mean factor of 0.457, and *hGPx1* by a mean factor of 0.386 (Table 5). Comparison between non-smoking controls and cancer patients showed significant down-regulation of expression of all the selenoprotein encoding genes in leukocytes in patients with bladder cancer, while cancer patients who smoked presented with significantly lower expression of *hGPx1* and *hSep15* compared with controls that smoked. GPx3 activity was significantly lower in both non-smoking and smoking patients with cancer compared to their respective control groups (Table 5). The observed correlations between the expression level of all the selenoprotein mRNAs in the control group were almost entirely related to non-smokers (Table 6).

Discussion

Se and selenoproteins are widely implicated in the aetiology of cancer, suggesting several hypothesis for the anticarcinogenic activity of this trace element. Se and selenoprotein concentrations in blood or blood compartments were lower in cancer patients than in controls (4). The mechanism of the anticarcinogenic activity of Se in bladder neoplasms can be explained by a possible direct and indirect effect on bladder mucosa, via plasma, reducing the concentration of carcinogens. In this study, plasma Se concentration and GPx1 activity did not differ in patients with bladder cancer and controls.

Data concerning selenoprotein mRNA expression in malignant and non-malignant tissue still provides insufficient information about bladder tumours. There are no good data on the levels of different selenoprotein transcript concentrations in tumours and benign

Table 5 Differences in selenoprotein mRNA expression normalised to *hGAPDH*[a], selenoprotein mRNA relative expression[b] and selenium status in patients with bladder cancer and controls with respect to smoking status.

	Non-smokers, n=34 Mean±SD	Smokers, n=13 Mean±SD	p-Value[a]	Expression ratio (SE)	p-Value[b]
Controls					
Se, ng/mL	56.0±12.9	50.0±18.23	0.069		
GPx1, U/g Hb	22.4±3.3	24.2±4.4	0.094		
GPx3, U/L plasma	0.200±0.041	0.201±0.037	0.943		
hGPx1	0.813±0.732	0.342±0.508	<0.0001	0.386 (0.201–1.243)	<0.0001
hGPx3	0.0031±0.0031	0.0012±0.0016	0.0002	0.457 (0.238–1.878)	<0.0001
hSep15	0.293±0.162	0.240±0.0350	0.0001	0.645 (0.456–0.914)	<0.0001
hSeP1	0.0042±0.0053	0.0053±0.0100	0.529	0.934 (0.465–2.018)	0.716
	Non-smokers, n=20 Mean±SD	Smokers, n=13 Mean±SD	p-Value[a]	Expression ratio (SE)	p-Value[b]
Cancer					
Se, ng/mL	48.6±22.3	53.5±24.6	0.672		
GPx1, U/g Hb	22.1±6.6	21.9±8.4	0.839		
GPx3, U/L plasma	0.172±0.035[c]	0.164±0.038[c]	0.495		
hGPx1	0.343±0.089[c]	0.497±0.321[c]	0.246	1.252 (0.660–2.380)	0.300
hGPx3	0.0015±0.0010[c]	0.0012±0.0009	0.387	0.924 (0.265–2.508)	0.847
hSep15	0.156±0.069[c]	0.200±0.087[c]	0.191	1.526 (0.809–1.654)	0.239
hSeP1	0.0018±0.0025[c]	0.0020±0.0014	0.146	1.356 (0.781–2.746)	0.230

[a]Reference gene-normalised relative quantification with efficiency correction; [b]calibrator-normalised relative expression; [c]statistically significant differences ($p < 0.05$) between controls and cancer patients. Hb, hemoglobin.

Table 6 Correlation between selenoprotein mRNA expression normalised to *hGAPDH* from leukocytes of controls in relation to smoking status.

Correlation	Non-smokers, n=34		Smokers, n=13	
	r	p-Value	r	p-Value
hGPx1/hGPx3	0.651	<0.0001	0.388	0.190
hGPx1/hSep15	0.559	0.0006	0.418	0.155
hGPx1/hSeP1	0.508	0.002	0.226	0.459
hGPx3/hSep15	0.614	0.0001	0.720	0.006
hGPx3/hSeP1	0.560	0.0006	0.187	0.541
hSep15/hSeP1	0.775	<0.0001	0.264	0.384

samples. It is presumed that tissue Se status, as well as genetic and epigenetic alterations such as deletions and hypermethylation can influence mRNA concentrations of selenoproteins (18, 19). Our findings may support the hypothesis that there is low expression of selenoprotein mRNAs in neoplasms. We found that leukocytes from patients with bladder cancer showed significant down-regulation in *hGPx1*, *hGPx3*, *hSep15* and *hSeP1* expression compared to leukocytes from controls. The results from patients with TCC of the urinary bladder showed up-regulation of tissue GPx in cases with different grades of tumour and invasive characteristics (20). Our findings concerning *hGPx3* and *hSep15* expression in leukocytes of patients with bladder cancer, which correlated with tumour grade, add to the data concerning expression of selenoprotein genes in peripheral leukocytes of patients with different grades of bladder TCC.

It is well known that selenoproteins are unevenly distributed amongst different tissues. The most ubiquitous is GPx1, Sep15 is found primarily in the liver and prostate, kidney is the main source of GPx3 and SeP is synthesised in the liver. Different selenoprotein mRNA expression levels may be associated with the hierarchy of selenoproteins in response to Se status (8). The present study indicates that there is a large inter-individual variation in selenoprotein gene expression in human circulating leukocytes with specific expression patterns. The observed higher expression of mRNA of *hGPx1* and *hSep15* in leukocytes compared with *hGPx3* and *hSeP* may also suggest a first-line of action for GPx1 and Sep15 in antioxidant defences in WBCs. Inter-individual differences in selenoprotein expression in leukocytes, observed in our study, can be associated with heterogeneous WBC populations used in this investigation, including lymphocytes, monocytes, and granulocytes. Each subset of leukocytes presents a unique pattern of gene expression related to its specific function (21). Different expression of selenoprotein mRNAs in patients with cancer of the urinary bladder and controls may reflect the relative proportions of specific WBC subpopulations. In addition, the results of our study did not confirm a functional significance for *GPx1* Pro198Leu and *Sep15* 1125G/A genetic polymorphisms, which could potentially modify Se status and/or *hGPx1* and *hSep15* expression in peripheral leukocytes. To date, genetic polymorphisms within

selenoproteins have been found in *hGPx1*, *hGPx3*, *hSep15*, and *hSeP1* genes. The functional effect of these polymorphisms was confirmed primarily in studies of cell cultures (10).

Some studies have indicated that specific gene transcription in circulating blood leukocytes is associated with cigarette smoking (13, 22, 23). It has been observed that smoking can reduce Se concentrations within the body, due to possible interactions with arsenic that is present in tobacco smoke (23). We did not find significant differences in plasma Se concentrations, RBC GPx1 or plasma GPx3 activity between non-smokers and smokers with bladder cancer and controls. In TCC of the bladder, there were no differences in selenoprotein gene expression between non-smokers and smokers. However, in the control group, *hGPx1*, *hGPx3* and *hSep15* showed significantly higher expression in leukocytes from non-smokers compared with expression leukocytes from smokers. Moreover, significant correlation between expression levels of all the studied selenoprotein mRNAs were generally seen in non-smoking healthy controls.

A growing number of studies have investigated different populations of WBC transcriptome patterns. This readily accessible human biological material can be easily used and is supposed to be a biomarker of nutritional status (24, 25). The study performed on rats by Evenson et al. (12) indicated that WBC *rGPx1* mRNA concentrations can be a useful biomarker of the effects of Se exposure. However, our results did not confirm the hypothesis that the expression of selenoprotein mRNA in circulating leukocytes can reflect Se status. Correlation between mRNA *hGPx1*, *hGPx3*, *hSep15*, *hSeP1* and Se status showed no significant association in patients with TCC of the urinary bladder or healthy controls. Recent results by Ravn-Haren et al. (23, 24) also indicated lack of alterations in selenoprotein mRNA expression in WBCs after short- and long-term supplementation with Se. Following one week of non-organic or organic forms of Se supplementation in healthy young Danish men, there were no differences in expression of *GPx1*, cytosolic *TrR1* and *SeP1* in leukocytes.

In conclusion, significant differences in expression of *hGPx1*, *hGPx3, hSep15* and *SeP1* in leukocytes of patients with bladder cancer when compared with controls appear to be associated with smoking status. Moreover, in non-smoking controls, a significant positive relationship between expression of all the studied genes was observed. We did not find a functional significance for GPx1 and Sep15 genetic polymorphisms with respect to Se status or gene expression in leukocytes. Transcript concentrations in circulating leukocytes of bladder cancer patients and controls did not reveal a potential impact of Se status on expression of selenoproteins. Differences in age and the relatively low number of study participants were limitations of our study. Despite this, the use of WBCs, including leukocytes, warrants further investigations in a larger group of patients of both genders with different baseline Se status, and in patients with different types of neoplasms. The elucidation of the potential interactions between selenoprotein transcript concentrations and selenoprotein concentrations and activity, in relation to several genetic polymorphisms of selenoproteins, should be also included.

Conflict of interest statement

Acknowledgements

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