Survival of Bladder or Renal Cancer in Patients With CHEK2 Mutations

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Survival of bladder or renal cancer in patients with CHEK2 mutations

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Abstract

Purpose: The purpose of this study was to compare the survival of CHEK2 mutations positive and CHEK2 mutations negative patients with bladder or kidney cancer.

Materials and methods: 1419 patients with bladder and 835 cases with kidney cancer and 8302 controls were genotyped for four *CHEK2* variants: 1100delC, del5395, IVS2+1G>A and I157T. Predictors of survival were determined among *CHEK2* carriers using the Cox proportional hazards model. The median follow-up was 17 years. Covariates included age (≤65; >66), smoking status (non-smoking; smoking), cancer family history (negative; positive) and gender (females; males).

Results: Of the 1419 bladder patients enrolled in the study, 118 (8.32%) carried a *CHEK2* mutation (all variants combined) (OR=1.4; 95% CI 1.17–1.78; *p*=0.0006), including 25 (1.76%) cases with a truncating mutation (OR=1.84; 95% CI, 1.17-2.89; *p*=0.01) and 93 (6.55%) patients with a missense mutation (OR=1.35; 95% CI, 1.07-1.7; *p*=0.01). We found no impact of *CHEK2* mutations on bladder or kidney cancer survival. The 10-year survival for all *CHEK2* mutation for bladder cancer carriers was 19% and for non-carriers was 13% (*p*=0.7). The 10-year survival for kidney cancer carriers was 6% and for non-carriers was 4% (*p*=0.9).

Conclusion: We found no impact of *CHEK2* mutations on bladder or kidney cancer survival regardless of their age, sex, cancer family history and smoking status.

Keywords: Mutation; *CHEK2*; Survival.

Introduction

Mutations in the cell cycle checkpoint kinase 2 (*CHEK2*) tumor suppressor gene are associated with multi-organ cancer susceptibility including cancers of the breast, prostate, bladder, kidney, thyroid, gastric and colon [1-4,10-16]. In Poland there are three recurrent truncating mutations in *CHEK2* gene (1100delC, del5395, IVS2+1G>A) present in 1.0% and one common missense mutation (I157T) present in 4.9% of Polish population [1,3]. In 2004 we investigated 172 patients with cancer of bladder and 264 with kidney cancer. We have shown that frequency of the missense variant was significantly increased among cases with kidney cancer (9.8%; odds ratio OR 2.1; p=0.0006) [1]. In 2008, we studied 416 unselected cases of urothelial bladder cancer and the *CHEK2* mutations were found in 10.6% of the cancer cases (OR 1.9; p=0.0003) [4]. Recently we genotyped 835 patients with invasive renal cancer and 8302 adult controls. The missense mutation was present in 78 participants with renal cancer and 410 controls (9.3%; OR 2.0; p <0.001). A truncating mutations were present in 20 patients and 80 controls (2.4%; OR, 2.5; p=0.0003) [2]. To validate and extend our earlier findings herein we evaluated the prevalence of four commons *CHEK2* mutations among 1419 patients with bladder cancer. Additionally we evaluated the impact of these mutations on survival from 1419 bladder cases and 835 kidney cancer patients. To our knowledge no such study has been published up to now.

Material

1. Patients

This study includes 1419 unselected cases of urothelial bladder cancer (378 women and 1041 men) and 835 unselected kidney cancer (363 women and 472 men) diagnosed at the Urology Hospital in Szczecin between 1986 and 2018. A total of 1518 incident cases of bladder cancer and 869 kidney cancer were identified during the study period. Of these, 1419 patients with bladder and 835 with kidney cancer accepted the invitation to participate (93%; 96%). All patients had a histopathological diagnosis of cancer. The mean age of diagnosis of bladder cancer patients was 68,32 years (range 13–91) and 63,31 (range 17-91) of kidney cancer. A

family history was taken by the construction of family tree and the completion of a standardized questionnaire. A total of 46 patients with a family history of at least 1 bladder cancer in first or second degree relatives and 31 cases with a family history of at least 1 kidney cancer in first or second degree relatives were identified. Detailed information of smoking status was available for a subset of 1045 (74%) cases with bladder and 503 (60%) kidney patients (pack years). The vital status and the date of death of all of the cases were requested from the Polish Ministry of the Interior and Administration in February 2020, and were obtained in March 2020. In total we collected data of death of 730 (51%) patients with bladder and 216 (26%) kidney cancer. The study was approved by the Ethics Committee of Pomeranian Medical University in Szczecin.

2. Controls

The control group included 8302 cancer-free, population-based, adults from (the genetically homogeneous population) Poland. In order to estimate the frequency of the Polish founder mutations in the general population, two control groups were combined. The first control group included 3,956 cancer-free men age 23–90 years (mean age 61.2 years) unselected for family history. The second control group consisted of 4,346 cancer-free females aged 19–91 years (mean age 52.2 years) unselected for family history. These controls are described in detail elsewhere, male controls [9] and female controls [8]. The allele frequencies for all variants in our control group were not dependent on age or sex, and the prevalence estimates of mutations in all genes were similar in younger and in older controls.

Methods

DNA was isolated from 5 to 10 mL of peripheral blood. The three mutations in *CHEK2* (1100delC, IVS2+1G>A and I157T) were genotyped as described previously [1]. In brief, these variants are detected by ASO- or RFLP-PCR analyses. The third truncating mutations in *CHEK* del5395 were genotyped as described previously and was detected by a multiplex PCR reaction [3]. In all reaction sets, positive and negative controls (without DNA) were used. All PCR reactions or enzymatic digestions were performed under a layer of mineral oil. Duplicate genotyping for quality control was performed for 382 randomly selected individuals, but no discrepancies with the initial results were found. As a further check, all mutation-positive cases were confirmed by sequencing, but again with no discrepancies.

Statistical analysis

1) Survival analysis

For the survival analysis, the patients were followed from the date of diagnosis of bladder or kidney cancer until death or March 2020. The median follow-up was 204 months. Survival curves were calculated by Kaplan-Meier analysis and Log rank test. Comparison of survival between carriers of a *CHEK2* mutation and non-carriers was performed by Cox regression analysis. Covariates included age (≤65; >66), smoking status (non-smoking; smoking), cancer family history (negative; positive) and gender (females; males).

The survival analysis was first performed using all subjects and then on the subgroups of individuals divided according to: age, sex, smoking status and cancer family history. The effect of carrying a *CHEK2* mutation was modeled first for all mutations and then separately for missense and truncating mutations.

2) Odds ratios

The prevalence of each of the four *CHEK2* alleles was compared in bladder cancer cases and in controls. The three protein truncating mutations were studied separately from the missense variant. Odds ratios were generated from two-by-two tables and statistical significance was assessed with the Fisher exact test where appropriate. The odds ratios were used as estimates of relative risk and additionally were adjusted for age, sex and pack-years of smoking by multiple logistic regression

Ethical statement

The study performed in accordance with the principles of the Declaration of Helsinki. All patients and controls provided written informed consent.

Results

1. Bladder cancer

Of the 1419 bladder patients enrolled in the study, 118 (8.32%) carried a *CHEK2* mutation (all variants combined) (OR=1.4; 95% CI 1.17–1.78; *p*=0.0006), including 25 (1.76%) cases with a truncating mutation (OR=1.84; 95% CI, 1.17-2.89; *p*=0.01) and 93 (6.55%) patients with a missense mutation (OR=1.35; 95% CI, 1.07-1.7; *p*=0.01).

The study subjects were followed from the date of diagnosis until death or March 2020 (a mean of 34 years). There were 51 deaths (43%) recorded in 118 carriers of a *CHEK2* mutation compared with 679 deaths (52%) in 1,301 noncarriers (HR=0.9; 95% CI 0.70–1.15; *p*=0.4). There were 38 deaths (41%) among 93 carriers of missense mutation, and 13 deaths (52%) among 25 carriers of three truncation mutations.

None of the four analyzed mutations in the *CHEK2* gene had a significant role in the survival time of the patients with bladder cancer. Also none of the alterations had an effect on the survival rate, data was stratisfied for gender, smoking and family cases. The median survival was 98 months for patients with truncation mutations and 75 for patients with missense mutations compared to 79 months for non-carriers (HR=1.33; 95% CI 0.72-2.44; *p*=0.4, log-rank test), (HR=0.99; 95% CI 0.64-1.51; *p*>0.9, log-rank test) (Table 1). In the subgroup of patients with a truncating mutation, the 10-year survival was 23% and 18% for patients with missense mutations compared to 13% for non-carriers. After adjusting for age smoking status, family history or gender the HR for mortality associated with bladder cancer and *CHEK2* mutation was 0.93 (95% CI 0.68-1.28; *p*=0.7) for patients younger than 65 years old; 1.15 (95% CI 0.80-1.64; *p*=0.5) for cases older than 66 years old; 1.49 (95% CI 0.57-3.86; *p*=0.4) for non-smoking group; 0.87 (95% CI 0.54-1.39; *p*=0.6) for smoking patients; 1.11 (95% CI 0.87-1.41; *p*=0.4) for cases with no cancer family history; 0,50 (95% CI 0.12-2.09; *p*=0.3) for patients with positive cancer family history; 1.00 (95% CI 0.69-1.44; *p*=1.0) for females and 1.15 (95% CI 0.84-1.56; *p*=0.4) for males.

Table 1. Survival of patients with bladder cancer; by variant alleles of *CHEK2*

	Patients with truncation mutations (n=25)	**Patients with missense mutations (n=93)**	**Patients with any *CHEK2* mutation (n=118)**	**Patients with no mutation in *CHEK2* (n=1301)**
Median follow-	72	61	61	59

up (mo)				
Proportion of deceased (%)	52	41	43	52
Median survival (mo)	98	75	81	79
5-Year survival (%)	50	47	48	44
10-Year survival (%)	23	18	19	13
HR	1.33	0.99	1.08	1.0
95% CI	0.72-2.44	0.64-1.51	0.76-1.55	-
p-value	0.4	>0.9	0.7	-

Hazard ratio (HR), 95% confidence interval (CI), and p-values are calculated by coxph test. Data was stratified for gender, family and packs/year.

2. Kidney cancer

Data on survival were available for 835 patients with kidney cancer. The mean follow-up time was 34 years. There were 28 deaths (28%) recorded in 98 carriers of a *CHEK2* mutation compared with 188 deaths (25%) in 737 noncarriers (HR=1.08; 95% CI 0.85–1.36; p=0.5). There were 23 deaths (29%) among 78 carriers of missense mutation, and 5 deaths (25%) among 20 carriers of three truncation mutations.

None of the four analyzed mutations in the *CHEK2* gene had a significant role in the survival time of the patients with kidney cancer (Table 2). Also none of the alterations had an effect on the survival rate if the data was stratified for gender, smoking and family cases. The median survival was 83 months for patients with truncation mutations and 78 for patients with missense mutations compared to 50 months for non-carriers (HR=0.52; 95% CI 0.24-1.15; p=0.11, log-rank test), (HR=1.34; 95% CI 0.76-2.38; p=0.3, log-rank test) (Table 2). The 10-year survival was 8% for patients with missense mutations compared to 4% for non-carriers. No one patient with truncation mutations survive 10 years. After adjusting for age smoking status, family history or gender the HR for mortality associated with kidney cancer and *CHEK2* mutation was 0.91 (95% CI 0.69-1.21; p=0.5) for patients younger than 65 years old; 0.54 (95% CI 0.32-0.91; p=0.06) for cases older than 66 years old; 0.94 (95% CI 0.67-1.33; p=0.7) for non-smoking group; 0.91 (95% CI 0.61-1.38; p=0.7) for smoking patients; 0.94 (95% CI 0.73-1.21; p=0.6) for cases with no cancer family history; 0.20 (95% CI 0.03-1.54; p=0.1) for patients with positive

cancer family history; 0.89 (95% CI 0.62-1.28; p=0.5) for females and 0.92 (95% CI 0.66-1.30; p=0.6) for males.

Table 2. Survival of patients with kidney cancer; by variant alleles of *CHEK2*

	Patients with truncating mutations (n=20)	**Patients with missense (n=78)**	**Patients with *CHEK2* mutation (n=98)**	**Patients with no mutation in *CHEK2* (n=737)**
Median follow-up (mo)	57	100	95	66
Proportion of deceased (%)	25	29	28	25
Median survival (mo)	83	104	101	78
5-Year survival (%)	45	78	71	50
10-Year survival (%)	0	8	6	4
HR	0.52	1.34	0.9	1.0
95% CI	0.24-1.15	0.76-2.38	0.56-1.43	-
p-value	0.11	0.3	0.7	-

Hazard ratio (HR), 95% confidence interval (CI), and p-values are calculated by coxph test. Data was stratified for gender, family and packs/year.

Discussion

In this study, we found no impact of CHEK2 mutations on bladder or kidney cancer survival.

Recently Słojewski *et al.* suggested that mutations in *CHEK2* gene were significant risk factor for the number of recurrences [5]. They were watching the group included 24 *CHEK2* positive patients with bladder cancer and the control group consisted of 44 persons with superficial bladder cancer during 24-months. They analyzed the rate, risk of recurrence and free-recurrence survival. They found that *CHEK2* mutations correlated with the risk of recurrence (OR=6.47; p=0.08) and they are significant risk factor for the number of recurrences in follow-up. Spachmann *et al.* investigated the group of 126 bladder cancer patients and found that loss of immunohistochemical protein expression of *CHEK2* was associated with significantly worse progression-free survival (p=0.041) and in the high-risk groups with concomitant carcinoma in

situ (p=0.044), multifocal tumors (p<0.001) and tumor grading G3 (p=0.009) [6]. No correlation between *CHEK2* expression and recurrence-free survival and cancer-specific survival was found. Carlo *et al.* reported that *CHEK2* mutations were the most common inherited mutations found in 254 unselected patients with advanced renal cancer in the United States compared with the general population with an odds ratio of 3.0 (95% CI, 1.3-5.8; p=0.003) and 4 of 9 germline *CHEK2* mutants (44.4%) had LOH in the tumor [7].

In the literature there are some studies about the impact of *CHEK2* mutations on survival of patients with breast, prostate or pancreatic cancer. Huzarski *et al.* enrolled 3,592 women with stage I to stage III with breast cancer and did not find any association between survival of patients and mutation in the *CHEK2* gene. The 10-year survival of women with breast cancer and for all *CHEK2* mutation positive was 78.8% and for non-carriers was 80.1%. They only observed a statistically significant adverse effect of the missense mutation I157T on survival among women with ER-positive breast cancer (HR=1.53) [15]. In the study of Weischer *et al.* an elevated risk of breast cancer-specific death associated with a *CHEK2* truncating mutation was present for women with ER-positive breast cancers (HR=1.63). They found that *CHEK2* 1100delC heterozygosity in women with estrogen receptor-positive breast cancer was associated with a 3.5-fold risk of a second breast cancer a 1.6-fold risk of breast cancer-specific death and a 1.4-fold risk of early death [17]. The other investigator Muranen *et al* shown that missense mutation in *CHEK2* gene was not associated with increased risk of early death, breast cancer-associated death or distant metastasis relapse [18]. Cybulski *et al* examined 3750 men with prostate cancer and suggested that in terms of prognosis, the cancers in carriers of *CHEK2* mutations are not distinguishable from cancers in the population at large [19]. Goldstein *et al* observed that patients with pancreatic cancer and DNA damage repair gene mutations (*ATM*, *BRCA1/2*, *CDKN2A*, *CHEK2*, *ERCC4*, *PALB2*) had an improved overall survival as compared with patients without (16.8 vs. 9.1 months, p=0.03) [20]. However this study has been performed on a small number of cases (n=133).

Herein we found no impact of *CHEK2* mutations on survival from patients with cancer of bladder or kidney regardless of their age, smoking status, cancer family history and sex. Our results are consistent with finding published by Cybulski and Huzarski pointing at no association between *CHEK2* status and survival of breast and prostate cancer patients.

Declarations

Ethics approval and consent to participate

The study was approved by Ethics Committee of the Pomeranian Medical University in Szczecin, Poland. All participants gave informed written consent prior blood donating.

Consent for publication

Not applicable.

Data availability statements

Availability of data and materials are include in manuscripts

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