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The Prevalence of CHEK1 and CHEK2 Mutations in Prostate Cancer: a Retrospective Cohort Study

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ABSTRACT

Background: Prostate cancer (PCa) is one of the most common types of cancer among men. Mutations and accumulation of chromosomal deviations are correlated with the development and aggressiveness of PCa. Cell cycle checkpoint pathways and DNA repair mechanisms are reported to deviate in cancers. Mammalian checkpoint kinase 1/2 (*CHEK1*/*CHEK2*) genes act as key signal transducers inside the genomic integrity checkpoints. *CHEK1* and *CHEK2* gene mutations were reported in a few different types of cancers. In PCa, *CHEK2* mutations were studied, but *CHEK1* gene variations were not well investigated. **Objective:** This study aimed to investigate the occurrence of variations in the *CHEK1* and *CHEK2* genes in PCa in the Jordanian population. **Methods:** Formalin-fixed paraffin-embedded PCa specimens of radical prostatectomy surgical procedures from 74 Jordanian patients were subjected to DNA extraction, polymerase chain reactions and Sanger sequencing to screen the mutations in selected exons of *CHEK1* and *CHEK2* tumor suppressor genes. **Results:** The presence of F281L (T/C) (1.4%) homologous missense point mutation in the kinase domain of the *CHEK2* gene and P188P (1.4%) silent point mutation in the kinase domain of the *CHEK1* gene. In addition, the 1100delC mutation was not detected in the studied PCa specimens. **Conclusion:** In line with previous reports, the presence of *CHEK2* mutation with a frequency of 1.4% supported the possible role of genetic variants of this gene in the development of PCa. No 1100delC mutation was detected in this study. No association was found in this study between *CHEK1* mutations and the development of PCa. Further studies are needed with larger cohorts along with a screening of more exons in order to shed more light on the frequency of *CHEK2* gene mutations and their role in the development of PCa in Jordan.

Keywords: Prostate cancer, *CHEK1*, *CHEK2*, mutation, cell cycle.

1. BACKGROUND

Prostate Cancer (PCa) commonly affects older men and the incidences are increasing worldwide. GLOBOCAN estimates PCa as the third most common cancer with a 3.8% mortality rate worldwide among other cancers (1). In Jordan, prostate cancer was estimated as the fourth most commonly diagnosed cancer in men (7.9%) and the fourth cause of cancer-related deaths (2). Although many factors have been associated with the pathogenesis of PCa, including infectious agents, chemical toxicities, diet, ethnic origin and genetic predisposition, the etiology of PCa is still not fully understood (3, 4).

Genetic mutation landmarks have been reported to be associated with the development of PCa including recurrent mutations in *TP53*, *PTEN*, *BRCA1*, *BRCA2*, *SPOP*, *AR*, and *FOXA1* (5-10). These mutations have been found with variable prevalence in different populations; however, the identification of a molecular portrait of PCa is still an important issue in understanding its molecular pathogenesis. CHEK1 and CHEK2 are Serine/Threonine protein kinases, mediating the response of cells to DNA damage and regulating the essential molecular mechanisms of cell cycle progression, DNA replication, chromatin reorganization and apoptosis (11, 12). *CHEK1* and *CHEK2* gene mutations were reported in a few different types of cancers. For instance, *CHEK1* mutations have been detected in 28% of endometrial cancer, 10% of colon cancer, including non-polyposis colorectal cancers (13), and 9% of spo-

radic stomach tumors (14). However, it was not well investigated in PCa. Alternatively, different studies reported mutations in the *CHEK2* gene in different cancers such as those associated with Li-Fraumeni syndrome (15). Haruki et al reported a low frequency of somatic mutations in the *CHEK2* gene in small-cell lung cancer (16). As for breast cancer, some studies reported a high frequency of *CHEK2* mutations, while others found the contrary (17). *CHEK2* mutations were also reported in a subset of osteosarcomas (18). Moreover, a high frequency of *CHEK2* mutations was reported in papillary thyroid cancer (19), colorectal cancer (20), gastric cancer (21) and PCa (22-25). Certain *CHEK2* variants such as 1100delC and I157T were frequently reported in PCa (26). In particular, point mutations in the *CHEK2* gene (IVS2 + 1G>A or 1100delC) were identified in 1.6 % of PCa patients; however, these mutations were also detected in 0.5% of the control group (22). The results of another report showed the presence of *CHEK2* mutations in 4.8% of 578 PCa patients (24). In a comprehensive study, Wu et al identified different mutations in the *CHEK2* gene in 1.85 % of PCa cases and showed a significant impact of c.1100delC on lethal outcomes (23). These findings were supported by a large-scale meta-analysis study which showed that *CHEK2* 1100delC del (rs555607708) (A.A. 410) and I157T mutations are associated with the risk of PCa but not with familial PCa (25).

2. OBJECTIVE

This study aimed to investigate the occurrence of variations in the *CHEK1* and *CHEK2* genes in PCa in the Jordanian population.

3. MATERIAL AND METHODS

Subjects and Samples

The current retrospective study included 74 formalin-fixed paraffin-embedded (FFPE) prostatectomy tissue specimens from patients diagnosed with prostate cancer by anatomical pathologists at the Department of Pathology and Laboratory Medicine at King Abdullah University Hospital (KAUH) in Irbid, Jordan. The archived samples represented cases diagnosed between 2015 and 2018. This study was conducted and granted according to the provisions of the Human Ethics standard ethical approval to conduct this research by the human ethics committee at King Abdullah University/Jordan University of Science and Technology and Yarmouk University (IRB #: 13/1/881 and Hospital Policy: GM7601).

Genomic DNA Extraction

The FFPE tissues were obtained, and genomic DNA was extracted using DNA tissue extraction kits according to the manufacturer's protocol (NORGEN BIOTEK CORP, Canada; www.norgenbiotek.com). Briefly, four to six sections of FFPE tissue of each sample were collected in an Eppendorf tube and firstly deparaffinized by adding 1ml of xylene to each tube, mixed and incubated at 50°C for 5 minutes, subsequently centrifuged at 14,000 g for 2 minutes. Xylene was removed and 1ml of 100% ethanol was added, mixed and centrifuged at 14,000 g for 2 minutes, excess ethanol was removed and this step was repeated for a second time. The remaining tissue pellet was dried for about 10 minutes at room temperature. After that, deparaffinized tissue fragments were digested in 300 µl Digestion Buffer A and 10 µl of Proteinase K, then vortexed and incubated at 55°C for 1 hour, followed by 90°C for 1 hour. The suspension was mixed and 300 µl of Buffer RL was added and vortexed. 250 µl of 100% ethanol was added and vortexed. The lysate was moved to a spin column in a collection tube and centrifuged at 14,000 g for 1 min. The flowthrough was discarded, then 400 µl of Wash Solution A was applied to the column and centrifuged for 1 minute, the flowthrough was discarded and this step was repeated three times. Next to that, the column was spun for 2 minutes. Finally, the spin column was placed into a 1.7 ml Elution tube and 50 µl of Elution Buffer B was added to the column, and subsequently incubated at room temperature for 1 minute, then centrifuged at 14,000 g for 1 minute to elute the DNA. Then, the concentrations of DNA were measured nano-spectroscopically for purity, and the purified DNA was estimated for quality on 1% agarose gel electrophoresis (100 V, 20-30 minutes). Isolated DNA was stored at -20°C until use.

Polymerase Chain Reaction (PCR)

PCR amplification for the coding *CHEK1* and *CHEK2* genes targeting exons 6, 13 and 5, 12, respectively, according to the Ensemble database, was conducted using

Gene	Forward & Reverse Primers	Product Size	Tm
CHEK1ex6F CHEK1ex6R	5'- TTGCAAAACATTTTTATTCAGTGTC -3' 5'- CATGAATTCCTTGGTTTATTTCA-3'	322 bp	54°C
CHEK1ex13F CHEK1ex13R	5'- TTTTGTTTTTGTTTTTGTTTTGACA -3' 5'- ATTTGCAGTTTGCAGGACAG-3'	246 bp	60°C
CHEK2ex5F CHEK2ex5R	5'- TCTGCTATTCAAAGTCTGAAACAA -3' 5'- TCCTCCTATGAGAGAGTGGAAAA -3'	247 bp	56°C
CHEK2 ex12F CHEK2ex12R	5'- TGTCTTCTTGGACTGGCAGA -3' 5'- AGCCTGGACAACAGAGCAAG -3'	330 bp	60°C

Table 1. CHEK1 and CHEK2 primers with optimized annealing temperatures. Tm: Annealing temperature.

Clinicopathological data (n = 74)		
Age (years)	72	
Mean PSA (ug/L)	60.0	
Gleason Score	n	%
3+3	9	12%
3+4	18	24%
4+3	3	4%
4+4	10	14%
4+5	20	27%
5+4	3	4%
5+5	11	15%

Table 2. Clinicopathological data of the patients. PSA: Prostate-specific antigen.

specific primer sequences designed by Primer3 plus software (www.primer3plus.com), as summarized in Table 1. Briefly, PCR amplification was done in a 30µl reaction volume containing 3µl of genomic DNA, 0.3µM of forward-primer, 0.3µM of reverse primer, 20.4µl of nuclease-free water and 6µl of 5X master mix (5x HOT FIREPol® Blend Master Mix, Solis BioDyne, USA). The thermal cycler program was achieved in an XP CYCLER machine (Bioer, China) and set as 95°C/10 min, next by 40 cycles of 95 °C/30 sec, annealing temperature was optimized (Table 1) for 30 sec, 72 °C/30 sec, before the final extension step of 72 °C/5 min.

Agarose Gel Electrophoresis

PCR products were resolved on 1.5% (w/v) agarose gel containing 3µl ethidium bromide. After standard electrophoresis (100 V, 30-45 minutes), the running product was visualized under a transilluminator according to a 100-bp ladder (Quick load) for molecular size comparison to estimate the success of PCR.

DNA Sequencing

The purified PCR products were sequenced by an external service provider (GENEWIZ, NJ, USA). The output of sequencing was checked for quality by Finch TV 1.5 and analyzed by Mutation Surveyor software V5.1.2.

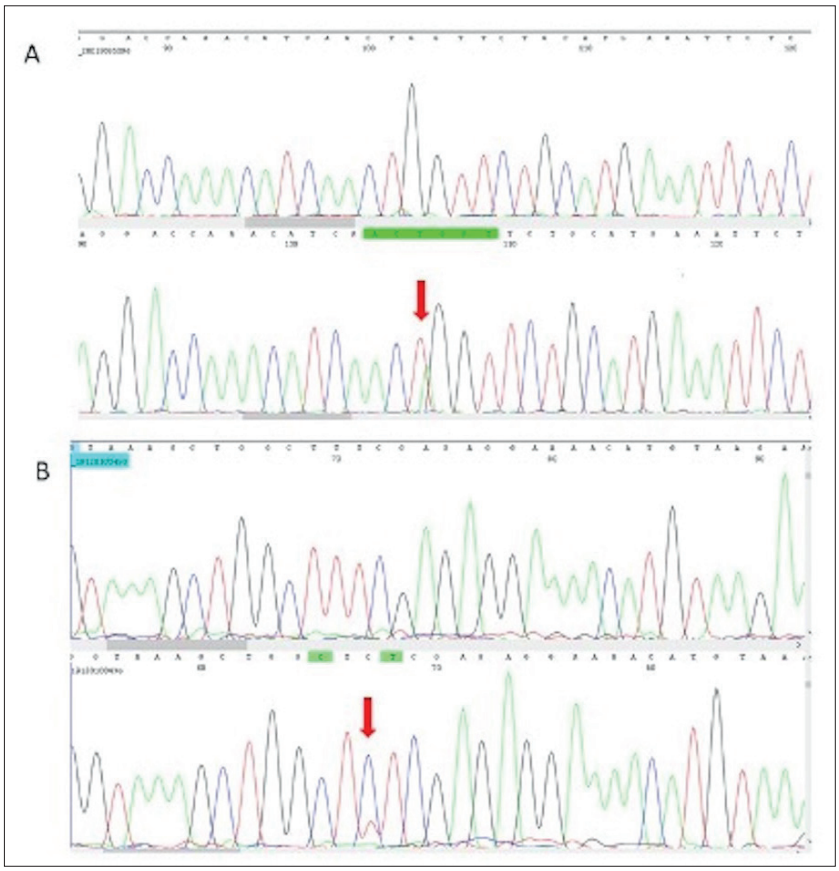

Figure 1. A representative chromatogram of point mutations in A) CHEK1 gene, c.564A>AT (188 P>P/P) and B) CHEK2 gene, F281L (T/C).

4. RESULTS

The study population included 74 FFPE samples with clinicopathological data as presented in Table 2. The mean age of the study population was 72 years, while the mean blood level of prostate-specific antigen (PSA) was 60 ug/L. The analysis of the *CHEK1* and *CHEK2* genes showed the presence of two point mutations in the *CHEK1* and *CHEK2* genes (2/74, 2.8%). Specifically, F281L (T/C) (1.4%) homologous missense point mutation in the *CHEK2* gene was reported in a 59 year old patient with a PSA level of 5.47 ug/L and a PCa Gleason score of (3+3). The second was a silent point mutation, c.564A>AT (188 P>P/P) (1.4%), in exon 6 (kinase domain) of the *CHEK1* gene (Figure 1).

5. DISCUSSION

CHEK1 and CHEK2 are mediators of DNA damage response, cell cycle progression, DNA repair mechanism and apoptosis process (11, 27, 28). In the present study, seventy four PCa cases were sequenced for the variations in checkpoint kinase genes (*CHEK1* and *CHEK2*). The results showed the presence of two point mutations in the two sequenced genes. In particular, one missense point mutation c.841T>C (F281L) was detected in the *CHEK2* gene and a silent mutation c.564A>AT (188 P>P/P) in the kinase domain of the *CHEK1* gene. The presence of these two mutations in PCa cases is at low frequency among Jordanian patients.

CHEK1 is conserved in different organisms including yeasts and mammals (29). Interestingly, *CHEK1* showed an essential role in embryogenesis in mice but not in yeast viability through the control of the G2/M damage checkpoint system (30). In addition, previous reports showed the role of *CHEK1* in the S-phase checkpoints (31). *CHEK1* is activated by kinase phosphorylation conducted by Rad3-related (ATR) protein (30, 32). An *in vitro* study showed that *CHEK1* is essential for cancer cell line progression but not for the normal cell line (33). These results were supported by the findings that showed a knockdown of *CHEK1* increases the radio-sensitization of the prostate cancer cell line (DU145) (34). Albiges et al reported a study on triple-negative breast cancer and showed an effective antitumor activity when targeting the CHEK1 protein (35). Alternatively, Stawinska et al found no significant expression of CHEK1 among colorectal cancer patients (36).

Through a certain extent of overlapping with the activation of *CHEK1*, *CHEK2* has been reported to play an important role in genome integrity and apoptosis in response to DNA damage. *CHEK2* gene mutation has been reported as a frequently mutated gene in Li-Fraumeni syndrome (37) and metastatic prostate cancer (38). In spite of the low frequency of *CHEK2* mutations in the current cohort, our results are consistent with the previous reports that support the possible association be-

tween CHEK2 mutations and the development of PCa. Wu et al reported in their study that almost 2% of PCa patients were *CHEK2* mutation carriers of different germline *CHEK2* mutations without an association with mortality or survival. The study reported c.1100delC as the most common variant in the tested population, which showed a possible contribution to PCa lethality (23). Additionally, CHEK2 showed significant expression in colorectal cancer (36). Bell et al suggested a possible role of *CHEK2* in cancer development rather than a tumor suppressor gene (39). The 1157T, 1100delC and 1422delT mutations in *CHEK2* are the most commonly reported mutations in different cancers, including Li-Fraumeni syndrome, breast cancer and prostate cancer (17, 39, 40). Collectively, most studies supported the association between the occurrence of *CHEK2* mutations/expression and the development of certain cancers. McPherson et al proposed a possible collaboration of *BRCA1* and *CHEK2* in cancer development (41). Moreover, a meta-analysis study showed that 1100delC and I157T mutations in *the CHEK2* gene are correlated with the susceptibility of PCa but not with inherited PCa (25). Therefore, targeting *CHEK2* can be suggested as an antitumor treatment option (42). Further studies supported the possible role of *CHEK2* mutations in the development of PCa. For instance, Dong et al reported an association between *CHEK2* mutations and sporadic prostate cancer (24). In another study, Cybulski et al found a significant correlation between the presence of 1100delC/ I157T and the risk of PCa in the Polish population (22). In a Finnish study, the researchers reported a significant association between the 1100delC mutation and hereditary prostate cancer (26).

6. CONCLUSION

In line with previous reports, the presence of *CHEK2* mutation with a frequency of 1.4% supported the possible role of genetic variants of this gene in the development of PCa. No 1100delC mutation was detected in this study. No association was found in this study between *CHEK1* mutations and the development of PCa. Further studies are needed with larger cohorts along with a screening of more exons in order to shed more light on the frequency of *CHEK2* gene mutations and their role in the development of PCa in Jordan.

Abbreviations

PCa: Prostate cancer
CHEK1: Checkpoint kinase 1
CHEK2: Checkpoint kinase 2
FFPE: Formalin-Fixed Paraffin-Embedded
PCR: Polymerase Chain Reaction

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- **Authors' contributions**: Mazhar Al Zoubi (MAZ), Mohammed Alorjani (MSA), and Manar Aburub (MA) designed and conceptualized the research idea. MSA, MA and SA collected patient data and samples. Manal AbuAlarja (MIA) and MA performed experiments. All authors analyzed and interpreted the data. MA, MAZ, MSA, BA and KA drafted the manuscript. All authors revised the manuscript critically and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.
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