RESEARCH ARTICLE

The *Fok1* Vitamin D Receptor Gene Polymorphism and 25(OH) D Serum Levels and Prostate Cancer among Jordanian Men

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Abstract

Background: Prostate cancer (PCa) is one of the most commonly diagnosed neoplasms and the second leading cause of cancer death in men in the Western world. Vitamin D (1,25dihydroxy vitamin D) is linked to many biological processes that influence oncogenesis but data on relations between its genetic variants and cancer risk have been inconsistent. The aim of this study was to determine associations between a vitamin D genetic polymorphism and 25-hydroxyvitamin D [25(OH)D] levels and prostate cancer. Materials and Methods: Genomic DNA was extracted from 124 Jordanian prostate cancer patients and 100 healthy volunteers. Ethical approval was granted from the ethical committee at Hashemite University and written consent was given by all patients. PCR was used to amplify the vitamin D receptor *Fok1* polymorphism fragment. 25(OH)D serum levels were measured by competitive immunoassay. Results: All genotypes were in Hardy-Weinberg equilibrium. Genotype frequency for *Fok1* genotypes FF, Ff and ff was 30.7%, 61.3% and 8.06%, for prostate cancer patients, while frequencies for the control group was 28.0%, 66.0% and 6.0%, respectively, with no significant differences. Vitamin D serum level was significantly lower in prostate cancer patients (mean 7.7 ng/ml) compared to the control group (21.8 ng/ml). No significant association was noted between 25(OH)D and VDR *Fok1* gene polymorphism among Jordanians overall, but significant associations were evident among prostate cancer patients (FF, Ff and ff : 25(OH)D levels of 6.2, 8.2 and 9.9) and controls (19.0, 22.5 and 26.3, respectively). An inverse association was noted between 25(OH)D serum level less than 10ng/ml and prostate cancer risk (OR 35.5 and 95% CI 14.3-88.0). Conclusions: There is strong inverse association between 25(OH)D serum level less than 10ng/ml level and prostate cancer risk.

Keywords: Prostate cancer - vitamin D - *Fok1 p*olymorphism - Jordan

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Introduction

PCa is one of the most commonly diagnosed forms of cancer among men in industrialized countries (Nwosu et al., 2001), whose incidence rates are rising rapidly in most countries including low-risk populations (Habuchi et al., 2000). It is a health problem in developed countries because of their greater proportion of elderly men. About 15% of male cancers are PCa in developed countries compared to 4% of male cancers in developing countries (Parkin et al., 2001). Prostate cancer incidence increases with age and it is estimated that 80% of men would be affected by the age of 80 years (Holund, 1980). In Jordan, PCa is the sixth among Jordanian male cancer which accounted for 7.9% of male cancers (www.moh.gov.jo).

Vitamin D (the sun-shine vitamin) plays a prominent role in bone and calcium metabolism also has functions in the immune system, central nervous system, epithelial cells, and various endocrine processes (Haussler et al., 1998). It has anticancer effects that are mediated through the vitamin D receptor (Brown et al., 1999), It promote cell differentiation, apoptosis, inhibition of cellular proliferation, angiogenesis and tumor cell invasion (Holt et al., 2009; Luong et al., 2010). VDR activation may regulate directly or indirectly the expression of 100-1250 genes (Yu and Cantorna, 2011; Zhang and Ho, 2011). Vitamin D exerts its biological effects through binding and activating the intracellular VDR, which acts as a ligand-dependent transcriptional factor in many types of tissues, including the prostate (Miller et al., 1992).

VDR is expressed in over 30 different cell types and located on chromosome 12q12-14 (Wu-Wong, 2007). Consisting of 14 exons and spanning approximately 75 kb long (Crofts et al., 1998). VDR gene encompasses two promoter regions, eight protein-coding exons (namely 2-9] and six untranslated exons (1a-1f) (Baker et al., 1998). Exons 2 and 3 of the VDR gene are involved in DNA binding, and exons 7, 8, and 9 are involved in binding to vitamin D (Hughes et al., 1988).

Polymorphisms of the VDR gene potentially affect the receptor binding Of 1, 25dihydroxyvitamin D3, that may modify vitamin D biological activity and confer

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susceptibility to prostate cancer (Yin et al., 2009). Oral administration of active vitamin D metabolites delays the recurrence of prostate cancer following primary therapy (Gross et al., 1998). This indicates that active vitamin D metabolites can be effective in slowing the progression of prostate cancer risk.

VDR gene is highly polymorphic and its allele frequencies are highly variable among different races and ethnic groups. More than sixty VDR polymorphisms have been discovered that are located in the promoter, in and around exons 2-9 and in the 3'UTRregion (Peehl et al., 1994). VDR gene variants that are studied include a 5' *Fok1* site in exon 2 that alters the start codon (Li et al., 1999). Correlation between low circulating levels of 25(OH)D were associated with an increased risk of subsequent earlier onset and more aggressive progression of prostate cancer, especially before the age of 52 (Polek and Weigel, 2002).

The aim of this study is to determine rs10735810 or *Fok1* polymorphism on exon 2 within VDR gene among prostate cancer Jordanian males. This polymorphism contain two potential translation initiation (ATG or start) sites (Saijo et al., 1991). A polymorphism has been described in the first start codon which changes the nucleotide sequence to ACG. The f allele contains both ATGs, whereas the F allele has only the second ATG, and thus predicts a shorter VDR protein (Ingles et al., 1998). This study also determined vitamin D level among prostate cancer patients and determine any association between v25(OH) D level and VDR*Fok1*gene polymorphism among prostate cancer patients.

Materials and Methods

A total of 124 prostate cancer patients were recruited from the urogenital cancer clinic at Al- Basheer Hospital/ Amman (2013-2014)that were histopathologically diagnosed with prostate cancer by specialized pathologists. One hundred age matched control volunteers with no familial history of any cancer were recruited from the Jordanian society. Ethical approval for this study was received from the Institutional Review Board (IRB) at the Hashemite University. Consent forms were signed by all participants before interviewing and sample collection. Plain tubes samples were centrifuged within two hours of sample collection, Serum was separated and stored at-60ºC for vitamin D determination. EDTA tubes were used for DNA extraction with in 2-4 hours from collection.

Almost 500µl of serum aliquots were used to measure serum 25(OH) D level using Elecsys vitamin D total assay kit (Roche Diagnostics, Switzerland) by MODULAR ANALYTICSE170 analyzer.

DNA samples were extracted using the Wizard Genomic DNA Purification kit (Promega, USA). DNA samples were amplified using the BIO RAD iCycler with the specific primers that are complementary to *Fok1* VDR gene

Forward 5'-ACTCTGGCTCTGACCGTG-3' and Reverse 5'-TCATAGCATTGAAGTGAAAGC. PCR was conducted using Go Taq® Green Master Mix DNA, then samples were amplified: Initial denaturation step at 94°C for 3 min, followed by 35 cycles of denaturation step at 94ºC for 90 sec then annealing step at 58ºC for 60s and extension at 72ºC for 90s. Finally the refrigeration cycle at 4ºC. Following amplification, SNPs FokI(rs2228570) in VDR gene was detected by restriction enzyme digest using the restriction endonuclease digestion (Jenna Bioscience, Germany) at 37°C for two hours. All fragments then visualized on 2% agarose gel electrophoresis. The expected size of *FokI* (FF) genotype is159 bp, FokI (Ff) genotype are159 bp+53 bp+106 bp and for FokI (ff) genotype are 53 bp+106 bp.

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 20.Chi-square test was used to evaluate case-control differences for FokI genotype distribution among case and control groups. T- test was used to evaluate the significance of difference of mean 25(OH)D levels between case and control groups. The association between FokI different genotypes and prostate cancer risk, vitamin D level and FokI different genotypes were evaluated by calculating the odd ratios (OR) using "Mantel Haenszel"method and 95% confidence intervals (CI).

Results

The genotypes and allele frequencies of FokI VDR gene polymorphism among prostate cancer and control participants are shown in table (1). The genotypes are in Hardy-Weinberg equation. There is no significant association of the VDR gene FokI gene polymorphism with prostate risk among prostate patients or healthy controls participants. The frequency of FF genotype was (30.7%) for prostate cancer patients compared with (28%) for healthy control. Ff genotype frequency in prostate cancer patients group was (61.3%) compared with (66%) for healthy control. ff genotype frequency was (8.1%) in prostate patients group compared to (6%) within the healthy control group (Table 1).

Our results (table 2) showed that the mean serum level of 25(OH)D for prostate patients (7.7±0.44 ng/ml) was significantly lower than the level in the control group (21.8

Table 1. Association of VDR Genotypic Frequencies among Prostate Cancer and Control Participants in Regard with Hardy-weinberg Equilibrium

Genotype	Case n(%)	Control n(%)	P value
FF	38 (30.7)	28 (28)	0.719
Ff	76 (61.3)	66 (66)	
Ff	10 (8.1)	6 (6)	
Allele			
F	152 (61.3)	122 (61)	0.95
F	96 (38.7)	78 (39)	

Table 2. Serum 25(OH)D Mean Levels among Prostate Cancer Patients and Control

	N	Mean±SE*(ng/ml)	p value
Prostate cancer patients	124	7.7±0.44	0.001**
Control	100	21.8±0.56	

*SE: Standard error of the mean. **p-value<0.05 is considered significant

Table 3. Association between 25(OH)D Level and Prostate Cancer Risk

(OH)D Status	Prostate Cancer Patients N=124	Controls n=100	OR	95% CI
25(OH)D less than 10.0 ng/ml	86 (69.35%)	6 (6.00%)	35.45	(14.28-88.03)
10.0 ≤ 25(OH)D ≥20 ng/ml	34 (27.42%)	77 (77.00%)	0.11	(0.06-0.21)
25(OH)D more than 20 ng/ml	4 (3.23%)	17 (17.00%)	0.2	(0.05-0.50)

*Deficient: less than 20ng/ml; Insufficient: between 10 and 20ng/ml; Optimal: more than 20ng/ml

Table 4. Serum 25(OH)D Mean Levels (ng/ml) for Each FokI Genotypes

Prostate Patients (n=124)				Control (n=100)			
VDR FokI Genotype	N	Mean ± Std (ng/ml)	p-value	VDR FokI Genotype	N	Mean ± Std (ng/ml)	p-value
FF	38	6.2±4.3	0.036	FF	28	19.0±5.5	0.002
Ff	76	8.2±4.2		Ff	66	22.5±5.1	
ff	10	9.9±9.2		ff	6	26.3±6.5	

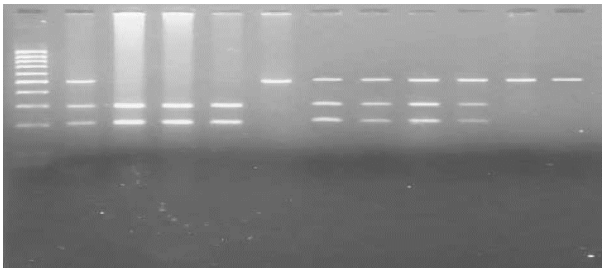

Figure 1. 2% Agarose Gel Electrophoresis for the Genotypes After FokI Enzyme Digestion of the PCR Product. Lane 1: 50bp DNA ladder. Lanes 2, 7, 8, 9, and 10: Ff genotypes. Lane 6, 11, 12: FF genotype. Lanes 3, 4 and 5: ff genotypes

ng/ml±0.56) (p-value=0.001).

The results of this study shows that there statistical significant difference in the mean 25(OH)D levels among FF, Ff and ff genotypes within both prostate cancer patients (p=0.036) and control (p=0.002)

Discussion

Vitamin D insufficiency affects almost 50% of the population worldwide. An estimated 1 billion people worldwide, across all ethnicities and age groups, have a vitamin D deficiency (Nair and Maseeh, 2012). Prostate cancer is one of the most common cancers among men, it is the second leading cause of cancer deaths worldwide (Siegel et al., 2013). Although it is less common in developing countries, its incidence and mortality rate is raised (Jemal et al., 2006). In Jordan and according to ministry of health at the Hashemite Kingdom ((www.moh.gov.jo)there were179 prostate cancer cases accounting 3.7% of all Jordanian cancer cases. Prostate cancer ranked the sixth among Jordanian male cancers which accounted (7.9%) of male cancers. Its etiology is unclear; however it may be related to ethnicity, environmental, genetics, hormonal and dietary factors (Tzonou et al., 1999; Lichtenstein et al., 2000)

The reason why VDR gene polymorphism has attracted attention because of the overall of anticancer effect of vitamin D itself. A number of studies have examined the role of VDR variants in prostate cancer with equivocal results ranging from statistically significant association (Oakley-Girvanet al., 2004; Jemal et al., 2006), weak association (Hayes et al., 2005) to no association (Guo et al., 2013) between common VDR variants and prostate cancer. Our finding showed no significant association of VDR *Fok1* gene polymorphism with prostate cancer risk. This is consistent with many previous studies (Yin et al., 2009; Zhang and Shan, 2013; Yousaf et al., 2014) among different ethnic groups.

Eighty three percent of the control participants in this study are deficient/insufficient for vitamin 25(OH) D level. This high percentage is similar to the percentage observed by Atoum and Tchoporyan (2014) among Jordanians. Although Jordanian climate may offers sufficient sunlight, the observed considerable deficiency and insufficiency might be attributed to other factors such as high pigmentation of Middle Eastern population, working indoors most of the daytime and dietary style (Nair and Maseeh, 2012).This study also shows that 17% of control have optimal serum 25(OH)D level (more than 20 ng/ml), while only 3% of prostate cancer patients are optimal. This study also shows that patients deficient in vitamin D (less than 10.0 ng/ml) had 35 fold increased prostate cancer risk compared to control. While increasing circulatory 25(OH)D level by adequate exposure to sunlight or oral supplementation promote the prostate cells to convert 25(OH)D to 1alpha 25(OH)D2 which has an antiproliferative effect in prostate cells (Donkena and Young, 2011). This finding shows that deficiency in 25(OH)D might participate in prostate cancer development and progression, and vitamin D level could be added as an additional factor to consider before ordering a biopsy for prostate cancer patients.

Our results showed significant difference in the mean of 25(OH)D level among various VDR *Fok1* genotype (FF, Ff, ff) within the prostate and control groups. Consitant with our results, a prospective study observed that the *Fok1* gene polymorphism interacted with 25(OH)D and modified prostate cancer risk in the presence of low 25(OH)D status (Li et al., 2007). On the other hand, Xu et al (2003) showed that presence of an F allele increased the risk of being diagnosed with more aggressive cancer because higher percentage of Gleason grade 4/5 is associated with worse prognosis. Huang et al (2006) suggested that the VDR *FokI* FF genotype increased the

risk of early-onset prostate cancer, especially its more aggressive forms (Huang et al., 2006). Our data showed that FF genotype associate with the lowest 25(OH)D among both prostate cancer patients and control (6.2 and 19), respectively. The frequencies of the different *Fok1* genotypes vary widely across different population and ethnic groups most likely due to different population`s diverse genetic behavior and exposure to mutagens leading to mutations that can amplify infrequency in a population.

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