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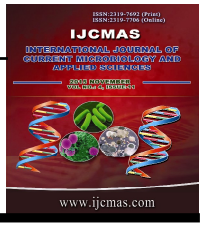


Original Research Article

Impact of *Fok-I* polymorphism of Vitamin D Receptor (VDR) Gene and Vitamin D Status in Bladder Cancer Incidence

Muhammed Abdul-Gabar Hamoodi Aldabagh[1*], Nidhal Abdul Mohaymen[2], Qasim Sh. Al-Mayah[1] and Ilham Ahmed Jasem[1]

[1]Medical Research unit/ College of Medicine/ Al-Nahrain University, Iraq
[2]College of Biotechnology/ Al-Nahrain University, Iraq
**Corresponding author*

Keywords

Bladder cancer incidence, Vitamin D, Fok-I polymorphism, Vitamin D receptor

A B S T R A C T

Bladder cancer is a prevalent disease with considerable correlated morbidity and mortality, it is the 9th (7th in men and 17th in women) most widespread cause of malignancy linked death globally. Aims of study is to investigation the association between vitamin D receptor (VDR) gene polymorphisms and serum levels of vitamin D (VD) with the incidence of bladder cancer (BC). A total of 59 patients with BC and 30 of healthy as control group were enrolled in this case-control study. Blood samples were collected from each participant. DNA extracted using commercial kit. and VDR gene were amplified by conventional PCR using specific primers. ELISA kit of VD used to estimate serum levels of VD. Logistic regression test revealed insignificant association between TT (ff) and CT (Ff) genotypes with the incidence of BC (OR=2.34, 95%CI= 0.044- 4.135, *p*=0.462 and OR= 1.84, 95%CI=0.055-5.379, *p*=0.601respectively). Serum levels of VD showed insignificant elevations in patient group (22.84 ± 0.93, P=0.11) compared to control group (20.25 ± 1.55). There was no association between Fok-I polymorphism and incidence of bladder cancer. In sufficiency status of vitamin D in both of cases and control groups could not help to confirm the negative relationship between VD deficiency and incidence of BC.

Introduction

Bladder cancer (BC) is a complex and multifactorial malignancy. It is the second most common genitourinary cancer (Parkin, 2004). Globally, BC is the ninth most common malignancy, over 330.000 new cases were estimated and over 130.000 estimated deaths yearly (Ferlay et al., 2008). Of note, the incidence of BC is higher in the developed countries such as European Union, Canada, and United states, while lower in the developing courtiers in Asia and Africa (Ploeg et al., 2009; Jamal et al., 2011). In Iraq, BC ranks 5th among ten most common malignancies in Iraqi population (Iraqi cancer registry, 2014). Although many individuals are exposed to same environmental factors, small ratio of them are develop BC, therefore genetic factors are

highly suggestive to play crucial role in development of such disease (Erol et al., 2015). In fact, accumulated evidences concluded that both genetic and environmental factors may jointly have an influence in the BC development (Anamaria et al., 2015). The classical actions of vitamin D (VD) on calcium and bone homeostasis (Geert_Carmeliet et al., 2015). Recently, the scientific outlook underscores the importance of VD in the maintenance of optimal health (Maladkar et al.,2015).

Anticancer activity of VD has been highly evaluated due to expression of VD receptor (VDR) by malignant cells, which focused attention toward the impact of VD in the protection and treatment of malignancy includeing its antiproliferative effect (Ingraham et al., 2008). The association of VDR gene polymorphisms and different type of malignancies has been investigated by a huge number of studies, which hypothesized that VDR gene polymorphism may impact both of risk and prognosis of cancer diseases (Kostner et al., 2009). Fok-I polymorphism of VDR gene has been reported to be associated with development of various type of cancer including bladder cancer (Mittal et al., 2007). In fact, studies on relationship of VDR gene polymorphism and development of BC are very limited, Moreover there is no such study in the middle East and Iraqi population.

Material and Methods

Five ml of venous blood was collected from each participant; 2 ml of which was kept in EDTA tube and the other 3ml in plan tube. The latter was undergone centrifugation where the serum was obtained and preserved at -20^{o}C until used for VD measurement using Human VD_3 ELISA kit, Cat. No. MBS291001 MyBiosource / USA . DNA was extracted from blood samples using ready kit (AccuPrep Genomic DNA extraction kit/bioneer/Korea). The ages of patients (males:49, females:10) ranged from 24 to 83years (males: 38 to 83years, females: 26 to 72years) and ages of controls (males:22, females:8) ranged from 51 to 80years (males: 51 to 80 years, females: 54 to 70years). The ages means± SD of studied groups were: cases 61.56±11.04 (males: 62.73±10.34, femals: 55.80±13.07), controls 61.03±8.88 (males: 60.55±9.18, females: 62.38±8.45). Informed consents from patients as well as control were taken which included age, previous and current occupation, smoking, drinking, residence, previously infected with schistosomiasis and first relative family history of Bladder cancer as well as body mass index (BMI) and sun shine exposure (SSE). Ethical permission was obtained from all volunteer to collect samples and conduct this study. Selections of cases were accomplished with the assistance of urologists within such hospitals. The study was conducted in the medical research unit at College of Medicine-Al-Nahrain University. Extracted DNA from blood samples was used in PCR for amplification of *VDR* gene *Fok-I* SNP. A pair of primers specific for *VDR* gene *Fok-I* snp were used (F:5□-GATGCCAGCTGGCCCTGGCACTG-3□, R:5□-ATGGAAACACCTTGCTTCTTCT CCCTC-3□, Fragment:273pb). The PCR protocol was [Initial denaturation: 95^{o}C for 5 min, (Denaturation: 94^{o}C for 30 sec, Annealing: 60^{o}C for 30 sec, Elongation: 72^{o}C for 1 min) 40 cycles, Final elongation: 72^{o}C for 10 min].

Results and Discussion

Original data in the consent form involved age, previous and current occupation, drinking, residence, and family history of BC. However, all participants, but one BC patient, specified that they have never drunk, whereas, there was an extreme disparities in

occupations that make nonsense in statistical analysis. So these three factors have been dropped from the analysis. Statistical analysis of studied risk factors showed in Table-1. Statistical analysis showed significant differences between both genders within patients group (X^2=15.55, P=0.042, CI 95%, 0.016-0.109).Also showed a significant differences (P value=0.023) in the mean of BMI between group of BC patients (27.17±0.78) and controls group (30.12±0.90). Regarding SSE (hours/week), our data showed insignificant (P value=0.208) elevation in means of SSE in group of BC patients (9.99±1.50) compared to group of control. In the present study smoking status was categorized into three categories: never, ex-smokers and current smokers. Larger percentage of never smoked is among control group (73.33 against 57.62 %,), whereas, 16.66% of control are ex-smokers compared to 6.77% of BC patients, and 10% of control are current smokers compared to 35.59% of BC patients. Our data indicated that insignificant association was found among ex-smokers (P=0.055, OR=0.308, 0.09-1.02) and significant association among current smokers (P=0.030, OR=0.152, 0.03-0.83). Regarding schistosomiasis, our data revealed that 15 (25.42%) patients of BC were previously infected with schistosomiasis versus 44 (74.57%) patients were not infected (Table-). Pathological subtype revealed that 53(89.83%) cases were diagnosed as transitional cell carcinoma (TCC), while 6(10.17%) cases were diagnosed as sequamous cell carcinoma (SCC) (Table-2).

The statistical analysis for results of serum levels of VD showed insignificant elevations in total patient group (22.84 ± 0.93, P=0.11) compared with HC group (20.25 ± 1.55). The genotype was considered as homozygous wild type (*Fok-I* CC=FF) when only one band appears at the C=F allele. When two bands appeared, it was considered as homozygous variant (*Fok-I* TT=ff). The heterozygous variant genotype (*Fok-I* CT=Ff) was characterized by the appearance of three bands (figure-1). Restriction fragment length polymorphism of PCR products of *VDR*gene *Fok-I* SNP revealed three genotypes; CC=FF, CT=Ff, and TT=ff. In BC patients, the FF, Ff, and ff genotypes account for 29 (49.15%), 26(44.06%), and 4 (6.77%) respectively, compared to 17(56.66%), 12(39.99%), and 1(3.33%) respectively, in control group (table).

Logistic regression test for the association of genotype with incidence of revealed insignificant association between CT genotype of *Fok-I* SNP in *VDR* gene and BC (OR=2.34, 95%CI= 0.044- 4.135, *P*=0.462). For the TT genotype represents 6.77% from the total genotype among BC patients compared with only 3.33% in control group, the difference between frequencies of this genotype is insignificant (OR= 1.84, 95%CI=0.055-5.379, *P*=0.601). (Table-1).

Allelic distribution

Using Chi-square for testing allele distribution, the result indicated that *Fok-I* SNP met Hardy-Weinberg equilibrium. Analysis of alleles frequencies of VDR rs2228570 SNP revealed insignificant differences in the frequency of C allele between BC patients and control (71.19% and 76.77% respectively), as well as frequency in allele T between the two groups (28.81% and 23.33% respectively, *P*=0.479). Also, statistical analysis for allelic distribution between males and females in patients revealed insignificant differences (X^2=0.912, P=0.424) (Table-2). Pathological subtypes of BC among patients revealed that 53 cases diagnosed as TCC (females: 10, males: 43) and 6 cases were

SCC all of whom were males (Table-4).

Table.1 Association of risk factors with the incidence of BC

Risk factor	Cases N=59	Control N=30	P value	OR(95%)
BMI (Mean±SE)	27.17±0.78	30.12±0.90	0.023	
SSE(Mean±SE)	9.99±1.50	7.17±0.94	0.208	
Smoking Never smoking EX-smoking Current smoker	 34(57.62%) 4(6.77%) 21(35.59%)	 22(73.33%) 5(16.66%) 3(10.0%)	 0.066 0.055 0.030	 1.0 0.308 (0.09-1.02) 0.152 (0.03-0.83)

BMI: body mass index, SSE: sun shine exposure (hours/week), CI: confidence interval, OR: odds ratio, SE: standard error

Table.2 pathological subtypes and SBT among cases

Bladder carcinoma	Number = 59	Females		Males	
TCC	53(89.83%)	10		43	
SCC	6(10.17%)	0		6	
SBT	15(25.42%)	TCC=0	SCC=0	TCC=10	SCC=5
Non-SBT	44(74.75)	TCC=10	SCC=0	TCC=33	SCC=1

SBT: schistosomal bladder tumor. TCC: transitional cell carcinoma, SCC: seqamous cell carcinoma

Table.3 Genotypes of Fok-I SNP in patients and controls

Fok-I snp	Cases (59)		Control(30)		P value	OR	CI (95%)
	Females=10	Males=49	Females=8	Males=22			
CC=FF	29(49.15%)		17(56.66%)			1.0	
	6 (20.7%)	23 (79.3%)	3 (17.6%)	14 (82.4%)			
CT=Ff	26(44.06%)		12(39.99%)		0.46	2.34	0.044-4.135
	4 (15.4%)	22 (84.6%)	5 (41.7%)	7 (58.3%)			
TT=ff	4(6.77%)		1(3.33%)		0.60	1.84	0.055-5.379
	0(0%)	4(100%)	0(0%)	(100%)			

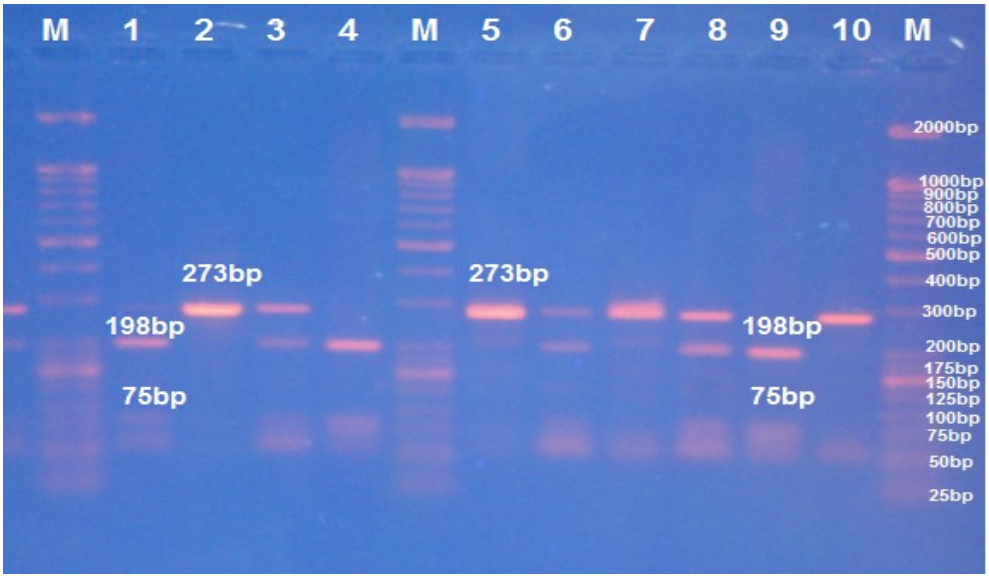
N: number, OR: odds ratio, CI: confidence interval, SNP: Single nucleotide polymorphism

Table.4 Allele frequency between patients and control for the SNPs Fok-I

SNP	Alleles	Cases	Control	Pearson X^2	P-value
Fok-I	C (wild)	84(71.19%)	46(76.77%)	0.607	0.479
	T(mutant)	34(28.81%)	14(23.33%)		

SNP: Single nucleotide polymorphism

Figure.1 Various *Fok-I* genotype patterns in BC cases observed after genotyping using RFLP-PCR. M: 25-2000bp DNA marker, lane 2, 5, 7, 10: wild type (CC=FF), lane 3, 6, 8: heterozygous variant (CT=Ff), lane 1, 4, 9 mutant (TT=ff)



Statistical analysis of current study did not shown any significant differences in VD levels between patients and control groups. In fact, according to scientific literatures, VD levels in studied groups occurred within insufficiency status.

Of note, accumulated epidemiologic and ecological data have linked VD deficiency with the incidence and mortality of many types of cancer. Increasing evidences, in vitro and in vivo animal model studies that revealed the anti-tumor effects of vitamin D (Chiang and Chen, 2013).

Naturally VD levels in different geographical regions never overreach 60ng/ml (150nmol/L) (Gilaberte et al., 2011). Other study suggested that VD deficiency, insufficiency, and deficiency were defined as VD levels less than 10ng/ml, less than 30ng/ml, and more than 30ng/ml respectively (Canadian Paediatric Society, 2007). However, Weinstock and Moses reported that more broadlly accepted that circulating levels of VD is 30-35ng/ml (75nmol/L) for optimal heath. Holick, 2004 and Giovannucci, 2005 reported that VD has been shown to stimulate cell differentiation as well as suppress proliferation angiogenesis, invasion, and metastasis of various cancers. Growing body of evidences that revealed a significant association between VD status and BC. In study on lab animals Konety *et al*. showed that VD is potent suppressor of proliferation of BC cells as well as bladder tumorgenesis. Mittal et al. suggested that elevation of VD levels may have a role in protecting against development of BC. In meta analysis study Peiris et al. reported that sufficient VD levels early in BC disease provide better chance to improve outcomes. In case control study Amaral et al. reported that VD deficiency was significantly associated with increased risk of BC (OR=1.83, 95% CI, 1.19-2.82, Pvalue=0.006). Different studies have revealed conflicting results, Michaud et al. found no relationship between VD intake and BC incidence. Also, Mondul et al.

observed insignificant association between VD status and risk of BC. Of note, Middle East has a hot and dry climate and the latitudes it spans, of 12° to 42° N, let synthesis of VD from ultraviolet B (UVB) rays for nearly all months of the year for more than 8 h /day (Holick, 2004). More broadly, it has been shown that VD status in the population of such area occur within in sufficiency status (Eggemoen et al., 2013; Fuleihan et al., 2009) as well as within elderly Iraqi population (Abdal Qader et al., 2012). Although previous studies attribute these results to poor nutrition, but this explanation may not be true for the fact that the samples taken from healthy people, especially in the current study were the exclusion of people with any chronic or acute diseases. However, the most accepted hypothesized of biological mechanism behind this conflicting results, is that because of ethnic differences and that includes variations on the genetic level and the consequences of the synthesis of proteins and mechanisms of activity especially with regard to the vitamin D metabolism. In other words, that vitamin D levels mentioned earlier (insufficiency) may need to carry out further local studies (Middle East) to identify private VD status among such population.

Present case control study showed insignificant association between both of Ff, ff variants and incidence of bladder cancer, or of incidence of Ff and ff variants among BC patients were 2.34 and 1.84 respectively. Growing body of evidence from many previous reports have shown that Ff and ff variants of *Fok-I* SNP are correlated with reduced transcriptional efficiency and may leads to more offensive disease prediction (Raimondi et al., 2009; Sweeney et al., 2006) . Alimirash et al. proposed that reason of efficiency of FF variant may be due to increased protein stability compared to ff variant.

Mishra et al. found significant association between f allele and incidence of breast cancer. Also, Mckay et al. found significant relation between ff variant and/ or f allele and development of breast cancer. In relation to colorectal cancer, Wong et al. found that ff variant associated with 2.5 fold elevated risk of colorectal malignancy that statistically significant. Mohapatra et al. studied the relationship between both of *Fok-I* variants and VD levels and development of ovarian cancer, they found that Ff and ff variants were correlated with 2 fold increased risk of ovarian cancer, and suggested that elevation risk of ovarian malignancy occur due to consolidation of VD deficiency and Ff, ff variants but not with FF variants.

In relationship of malignancy with ethnicity, meta analysis of huge number of studies conducted by Gnagnarella et al. found an extensive significant association of *Fok-I* variants together with each malignancy, with differential impact by ethnicity. Neill et al. assessed the impact of VD, ethnicity, and *Fok-I* variants on expression and efficiency of the VDR, they showed a significantly higher levels of VDR protein among African than whites, although VDR mRNA in whites was higher than in African. These results revealed a complex interaction among these three factors and elicited transactivation ability of definite target genes, which may illustrate diacordant genetic association of VDR with some diseases. The biological mechanism behind this inverse relationship between gene expression and levels of protein, propose that differential post transcriptional regulation between these ethnicities, and the mechanism of mRNA translation may vary between African and whites. Beside insignificant association of

Fok-I variants with BC incidence among BC patients (current study), Mital et al. investigated of such association and found significant differences in allelic distribution of Fok-I variants among BC patients, and FF variant was correlated with 2 fold risk of BC incidence (OR= 2,04, 95% CI, 0.803-5.193) [30].

In conclusion, our results support the hypothesis that VDR *Fok-I* variants may predict a low risk for BC incidence. VDR *Fok-I* polymorphism appears to be associated to BC disease in Indian study while not associated in current study may be attributed to small sample size in both studies as well as ethnicity differences between populations in both studies. Also may attributed to differences in both transcriptional regulations as well as mRNA translation mechanisms. Other factors may impact in BC incidence in consolidative or simultaneously manner. Further studies with prime study design are needed to confirm our results and investigate the hidden mechanisms underlying such relationship.

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Reference

Abdal Qader M.A.; Amin R.M.; Shah S.A. and et al. 2013. Relationship Between Nutritional Factors, Sports Practicing and Falls among Elderly People in Baghdad City, Iraq, Pakistan Journal of Nutrition 12 (5): 491-495.

Alimirah F,; Peng X.; Murillo G. and et al. 2011. Functional significance of vitamin D receptor FokI polymorphism in human breast cancer cells. PLoS One, 6(1):e16024.

Amaral A.F.; Méndez-Pertuz M.; Muñoz A. and et al. 2012. Plasma 25-Hydroxyvitamin D3 and Bladder Cancer Risk. J. Natl. Cancer Inst., Oct. 29.

Anamaria T.A.; George S.; Tudor-Adrian H. and et al. 2015. Etiopathogenesis of bladder cancer. Risk factors, genetic aspects and novel diagnosis biomarkers. HVM Bioflux, vol:7, issue: 3, p206-214.

Canadian Paediatric Society. 2007. Vitamin D supplementation recommendations for Canadian mothers and infants. Paediatr Child Health, 12:583-598.

Chiang K.C. and Chen T.C. 2013. The Anti-cancer Actions of Vitamin D. Anti-Cancer Agents in Medicinal Chemistry, 13, 126-139.

Eggemoen A.S.; Knutsen K.V.; Dalen I. and et al. 2013. Vitamin D status in recently arrived immigrants from Africa and Asia: a cross-sectional study from Norway of children, adolescents and adults. B.M.J. Open, 3: e003293.

Erol B.; Ismail U.; Yavuz O.D. and et al. 2015. Bladder cancer gene polymorphisms: A review. EMJ Urol. 3(1):20-25.

Ferlay J.; Shin H.R.; Bray F. and et al. 2010. Estimates of worldwide burden of cancer in 2008:GLOBOCAN 2008. Int J Cancer, 127:2893–2917.

Fuleihan G.E.H. 2009. Vitamin D Deficiency in the Middle East and its Health Consequences for Children and Adults. Clinic. Rev. Bone Miner Metab, 7:77-93.

Geert__Carmeliet G.; Dermauw V. and Bouillon R. 2015. Vitamin D

signaling in calcium and bone homeostasis: A delicate balance. Clinical endocrinology and metabolism, 2015, Article in press.

Gilaberte, Y. ; Aguilera, J. ; Carrascosa, J.M. and et al. 2011. Vitamin D: Evidence and Controversies. Actas Dermosifiliogr. 2011;102(8):572-588.

Giovannucci E. 2005. The epidemiology of vitamin D and cancer incidence and mortality: a review (United States). Cancer Causes Control, 16:83-95.

Gnagnarella P.; Pasquali E.; Serrano D. and et al. 2014. Vitamin D receptor polymorphism *FokI* and cancer risk: a comprehensive meta-analysis. Carcinogenesis, vol.35 no.9 pp.1913–1919.

Holick MF. 2004. Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. Am. J. Clin. Nutr., 79: 362–71.

Ingraham B.A.; Bragdon B. and Nohe A. 2008, Molecular basis of the potential of vitamin D to prevent cancer. Current Med. Res. Opin., 24:139–149.

Iraqi cancer registry 2011: Baghdad-Iraq 2014.

Jamal R, Bray F, Center M, et al. 2011. Global Cancer Statistics. CA Cancer J Clin, 61, 69-90.

Konety B.R.; Lavelle J.P.; Pirtskalaishvili G. and et al. 2001. Effects of vitamin D (calcitriol) on transitional cell carcinoma of the bladder in vitro and in vivo. J. Urol., 165:253-8.

Kostner K.; Denzer N.; Muller C.S.L. and et al. The Relevance of Vitamin D Receptor (VDR) Gene Polymorphisms for Cancer: A Review of the Literature. Anticancer Research, 2009, 29: 3511-3536.

Maladkar M.; Sankar S. and Kamat K. 2015. Vitamin D Efficiency in Pregnancy: An Updated Viewpoint in Indian Scenario. International Journal of Clinical Medicine, 6, 204-216.

McKay J.D.; McCullough M.L.; Ziegler R.G. and et al. 2009, Vitamin D receptor polymorphisms and breast cancer risk: results from the national cancer institute breast and prostate cancer cohort consortium. Cancer Epidemiol. Biomarkers Prev., 18: 297–305.

Michaud D.S.; Spiegelman D.; Clinton S.K. and et al. 2000. Prospective study of dietary supplements, macronutrients, micronutrients, and risk of bladder cancer in US men. Am. J. Epidemiol., 152:1145–53.

Mishra D.K.; Wu Y.; Sarkissyan M. and et al. 2013. Vitamin D Receptor Gene Polymorphisms and Prognosis of Breast Cancer among African-American and Hispanic Women. PLOS ONE March, Volume 8, Issue 3, e57967.

Mittal R.D.; Manchanda P.K.; Bhat S. and et al. 2007. Association of vitamin-D receptor (Fok1) gene polymorphism with bladder cancer in an Indian population. B.J.U. Int., 99: 933-937.

Mohapatra, S.; Saxena, A.; Gandhi, G. and et al. 2013. Vitamin D and VDR gene polymorphism (FokI) in epithelial ovarian cancer in Indian population. J. OvarianRes.2013, 6, 37.

Mondul A.M.; Weinstein S.J.; Horst R.L. and et al. 2012. Serum vitamin D and risk of bladder cancer in the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial. Cancer Epidemiol Biomarkers Prev. 21(7): 1222–1225.

Neill V.; Asani F.F.; Jeffery T.J. and et al. 2013. Vitamin D Receptor Gene Expression and Function in a South

African Population: Ethnicity, Vitamin D and FokI. PLOS ONE , Volume 8 , Issue 6 , e67663.

Parkin D.M. 2004 International variation.Oncogene, 23: 6329–40.

Peiris A.N.; Bailey B.A. and Manning T. 2013. Relationship of vitamin d monitoring and status to bladder cancer survival in veterans. South Med. J., 106(2):126-30.

Ploeg M, Aben K, and Kiemeney L. 2009. The present and future burden of urinary bladder cancer in the world. World J Urol, 27, 289-93.

Raimondi R.; Johansson H.; Maisonneuve P. and et al. 2009. "Review and meta-analysis on vitamin D receptor polymorphisms and cancer risk," Carcinogenesis, vol. 30, no. 7, pp. 1170-1180.

Sweeney C.; Curtin k.; Murtaugh M.A. and et al. 2006."Haplotype analysis of common vitamin D receptor variants and colon and rectal cancers," Cancer Epidemiology Biomarkers and Prevention, vol. 15, no. 4, pp. 744-749.

Weinstock M.A. and Moses A.M. 2009. Skin cancer meets vitamin D: the way forward for dermatology and public health. J. Am. Acad. Dermatol., 61:720-4.

Wong H.L.; Seow A.; Arakawa K. and et al. 2003. Vitamin D receptor start codon polymorphism and colorectal cancer risk: effect modification by dietary calcium and fat in Singapore Chinese. Carcinogenesis, vol.24 no.6 pp.1091-1095.