Journal of Food and Nutrition Research, 2017, Vol. 5, No. 7, 475-480
Available online at http://pubs.sciepub.com/jfnr/5/7/4
© Science and Education Publishing
DOI:10.12691/jfnr-5-7-4

Association of Vitamin D Receptor *Fok*I and *Taq*I Gene Polymorphisms in Pakistani Women with 25(OH)D Levels

Amna Younus[1,*], Mariam Faiz[2], Abida Yasmeen[1]

[1]Department of Chemistry, Lahore College For Women University
[2]Department of Pathology, Institute Of Nuclear Medicine And Oncology Lahore
*Corresponding author: hayya_pak@yahoo.com

Abstract Vitamin D receptor mediates the effects of calcitriol on gene expression. VDR gene polymorphisms have been linked with specific health outcomes, including increased risk of osteoporosis, cardiovascular disease, diabetes, some cancers (breast and prostate). Gene based studies give exceptional chance to associate molecular insights with epidemiological information and can disclose factual biological effects. The purpose of present study was to evaluate the genotypic distribution of *Fok*I and *Taq*I polymorphisms of the VDR gene and their association with serum vitamin D levels in healthy Pakistani women by PCR-RFLP technique. Three hundred healthy women between the age of 19-75years; were selected from different areas of Punjab province, Pakistan. Results showed that genotypic frequency for FF, Ff , ff, TT, Tt and tt was 55, 3, 42, 15, 65, and 20% whereas allelic frequency F vs f and T vs t was 56 vs 44% and 47.5vs 52.5% respectively in Pakistani women population. Results of the study concluded that genotypic frequency distribution of *Fok*I and *Taq*I polymorphisms in Pakistani population varies differently from other populations and both of these SNPs are associated with vitamin D deficiency and insufficiency. The data may provide basis for future clinical and epidemiological studies.

Keywords: *VDR gene polymorphism, FokI, TaqI, healthy women, Punjab Pakistan*

Cite This Article: Amna Younus, Mariam Faiz, and Abida Yasmeen, "Association of Vitamin D Receptor *Fok*I and *Taq*I Gene Polymorphisms in Pakistani Women with 25(OH)D Levels." *Journal of Food and Nutrition Research*, vol. 5, no. 7 (2017): 475-480. doi: 10.12691/jfnr-5-7-4.

1. Introduction

Vitamin D receptor (VDR) is a steroid hormone receptor and mediates the effects of 1,25-dihydroxyvitamin D on gene expression [1]. It is present in all body organs and tissues [2]. The VDR gene is of 5.6 kb and is located on chromosome 12 (12q12-14) [3] with at least five promoter regions, and is composed of at least 11 exons that span 60 kb of DNA [4] .VDR plays an active role for the transcription of various genes responsible for cell-cycle control, apoptosis, and metastatic potential by binding with its ligand (1,25(OH)2-D_3) [5,6,7] VDR gene have several polymorphisms. Most frequently reported SNP sites have been at the start codon (*Fok*I) and the 3′ untranslated region (*Taq*I) [1,8]. VDR gene polymorphisms have been linked with specific health outcomes, including increased risk of osteoporosis, cardiovascular disease, diabetes, some cancers (breast and prostate), and infectious diseases such as tuberculosis [9,10,11]. Our previous study have showed a high prevalence of vitamin D insufficiency in healthy pre and postmenopausal women [12]. Based on this study, it may be speculated that VDR gene polymorphisms could be linked to higher susceptibility to develop vitamin D deficiency. Therefore, the aim of the present study was to assess the genotypic distribution of *Fok*I and *Taq*I polymorphisms of the VDR gene and to determine whether these gene variants are associated with 25(OH)D levels in healthy Pakistani women population.

2. Methodology

2.1. Data Collection

The study was carried out at Institute of Nuclear Medicine and Oncology Lahore (INMOL). Three hundred healthy women between the age of 19-75years; from different areas of Punjab province, Pakistan were included in this study. Informed written consent was taken from each subject of the study. Approval of the study was obtained from scientific research review and ethical committee (registration no. 11-PhD/LCWU-20244) of the institution (Lahore College for Women University). Blood samples (5ml each) were collected by venipuncture and was further divided into parts of 3ml for estimation of serum vitamin D level and 2ml into EDTA vial for isolation of DNA. Vitamin D levels were estimated from serum samples of healthy women by using commercially available ELISA kit (Immunotech, Beckman Coulter Company, France). Participants of the study were categorized as vitamin D deficient on the basis of serum

25(OH)D concentrations of <20 ng/mL (50 nmol/L) and insufficient: 21 to 29 ng/mL (50 to 75 nmol/L) [13,14].

2.2. DNA Extraction

Genomic DNA was extracted from peripheral blood of healthy women using blood DNA extraction kit (Vivantis GF-1, Germany). Yield and purity of extracted DNA was determined by measuring absorbance at 260/280 nm. DNA samples were stored at -20℃ for further analysis.

2.3. *Fok*I Polymorphism

VDR gene amplification was performed by PCR in 25µl reaction mixtures containing primers for *Fok1* polymorphism (Harris et al., 1997 [15]. The PCR reaction mixture consisted of 25ng of genomic DNA, 2-6 pmol of forward and reverse primers, (Thermo Scientific). The PCR cycling conditions were initial denaturation at 94℃ for 5 min, followed by 35 cycles at 94℃ for 30 sec, 58℃ for 30 sec and 72℃ for 1 min and one final cycle of extension at 72℃ for 7 minutes. The PCR product (265 bp) was digested with 1.0 unit of Fok-I restriction enzyme (Thermo Scientific FastDigest enzyme) at 37℃ for 5 minutes. Then 10µl digested reaction mixture was loaded into 2% agarose gel containing ethidium bromide. The SNP at second nucleotide of exon 2 of VDR gene leading to T→C substitution resulted in the production of *Fok*I restriction site. Healthy women with homozygous alleles containing nucleotide T at this position showed an intact band of 265bp and designated as FF. Homozygous participants with alleles containing nucleotide C at second nucleotide of exon 2 confirmed 2 bands of 196 bp and 69 bp and designated as ff. Subjects in heterozygous category showed three bands of 265, 196, and 69 bp and were labeled as Ff [Figure 1].

2.4. *Taq*I Polymorphism

PCR amplification of VDR gene was done in 25µl reaction mixtures containing primers for *Taq1* polymorphism described elsewhere [16] The PCR amplification conditions were initial denaturation at 94℃ for 3 minutes followed by 35 cycles at 94°C for 30 sec, 62.8℃ for 35 sec, 72℃ for 45 sec and final extension at 72℃ for 10 minutes. The PCR product (454bp) was digested with 1.0 unit *TaqI* restriction enzyme (Thermo Scientific FastDigest enzyme) in a heat block at 65℃ for 5 minutes. Then 10µl digested product was resolved by 2% agarose gel containing ethidium bromide for *Taq*I restriction analysis. The SNP in VDR gene leading to T→C substitution at 1171 nucleotide in exon 9 resulted in the formation of *Taq*I restriction site. Homozygous alleles containing subjects with nucleotide T at this position showed a complete 454bp band and were labeled as TT. Homozygous alleles containing subjects with nucleotide C at the position of 1171 nucleotide showed 2 bands of 293 bp and 161 bp and were marked as tt. Heterozygous alleles containing participants showed 3 bands of 454, 293, and 161bp and were marked as Tt as shown in Figure 2.

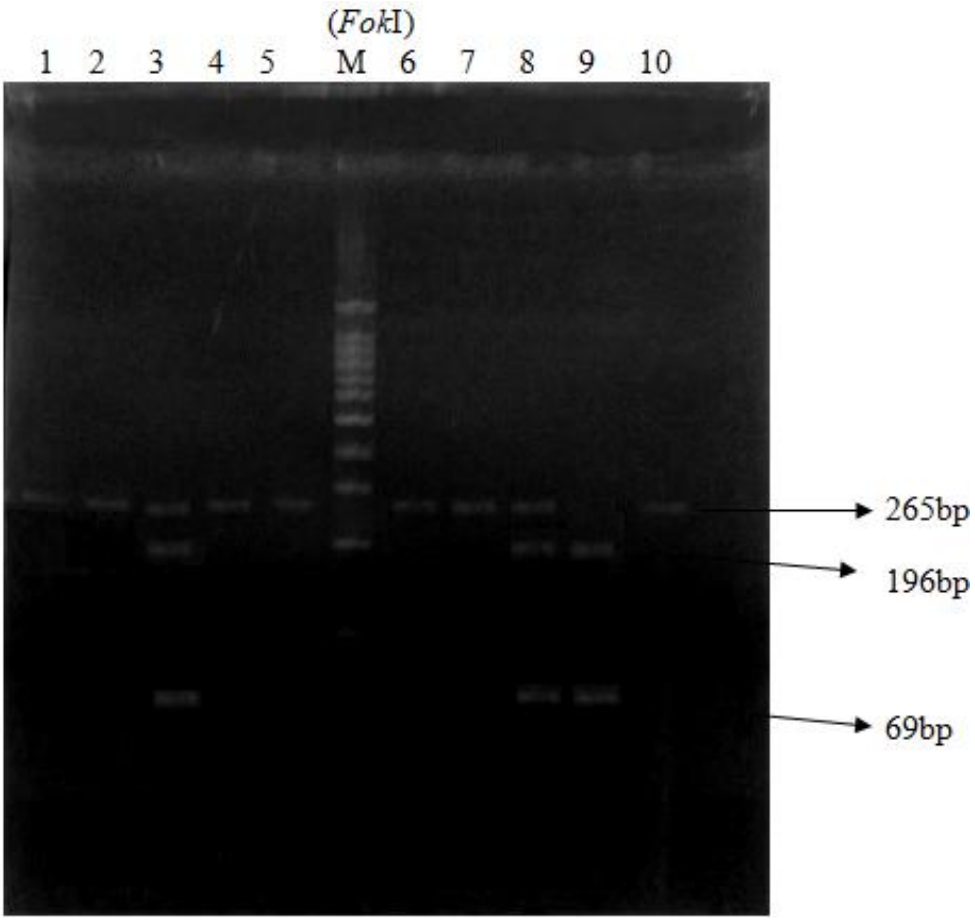

Figure 1. PCR-RFLP analysis of VDR gene. *Fok*I polymorphism on 2% agarose gel. M=marker (100bp ladder). Lane 1, 2, 4, 5, 6, 7, 10 represent 265bp bands (FF), Lane 9 represent 2 bands of 196 and 69bp (ff) and lane 3, 8 represent 2 bands of 265, 196 and 69bp (Ff)

2.5. Statistical Analysis

Genotype and allelic frequencies of the VDR gene polymorphism in healthy women were determined by Hardy-Weinberg equilibrium and association of *FokI* and *TaqI* polymorphism with vitamin D deficiency and insufficiency in healthy women was found by chi square test and *P* value <0.05 was considered significant.

3. Results

Out of 300 samples analyzed for *FokI* and *TaqI* polymorphisms by PCR-RFLP based analysis, the distribution of VDR genotypes (Figure 1, Figure 2) and allele frequency in Pakistani healthy women was shown (Table 1). The allelic frequency of 'F'; 'f ' was 56 and 44% and 'T' and 't' was 47.5 and 52.5% respectively and P-value= <0.00001 by chi^2 test clearly indicated a significant genotype distribution of VDR *Fok*I and *Taq*I polymorphisms in current population. The allelic frequency was found in agreement with Hardy-Weinberg equilibrium. Comparison of *Fok*I [Table 2] and *Taq*I [Table 3] frequencies of different populations with the present study by using X^2 test, showed a significant difference in genotype distribution of VDR (*Fok*I, *Taq1*) polymorphism in Pakistani healthy individuals.

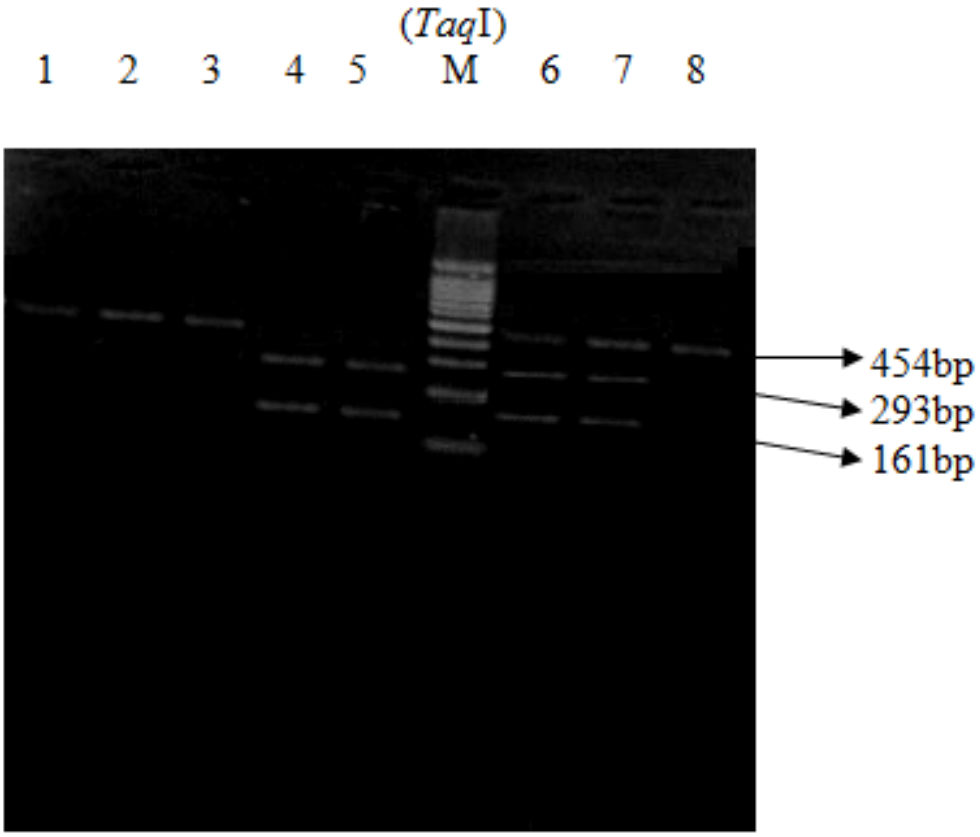

Figure 2. PCR-RFLP analysis of VDR gene. *Taq*I polymorphism on 2% agarose gel. M=marker (100bp ladder). Lane 1, 2, 3, 8 represent 454bp bands (TT), Lane 4, 5 represent 2 bands of 293 and 161bp (tt) and lane 6, 7 represent 3 bands of 454, 293 and 161bp (Tt)

Table 1. Genotypes and allele frequency distribution of VDR gene (FokI, *Taq*I) polymorphism in healthy women

Genotypes n=300 (%)			Allelic frequencies (%)	
FF	**Ff**	**ff**	**F**	**f**
163 (55)	10 (3)	127 (42)	56	44
TT	**Tt**	**tt**	**T**	**t**
45 (15)	195 (65)	60 (20)	47.5	52.5

Table 2. Comparison of genotypes frequency of VDR gene polymorphism (*Fok*I) between Pakistani and different populations

Population [ref]	Sample size	FF (%)	ff (%)	Ff (%)	X^2	*P*-Values
French [18]	100	43	10	47	11.03	0.004
Caucasians UK [19]	108	48	11	41	6.40	0.04
North Indians [8]	346	44	7	49	9.73	0.007
Japanese [20]	249	37	12	37	19.76	0.0001
Present study	300	55	42	3	260.78	<0.00001

Table 3. Comparison of genotypes frequency of VDR gene polymorphism (*Taq*I) between Pakistani and different populations

Population [ref]	Sample size	TT(%)	tt (%)	Tt (%)	X2	*P*-Values
French [18]	189	33	18	49	13.21	0.0014
Chinese [21]	144	90	0	10	59.23	0.0001
Japanese [20]	488	77	1	22	68.18	0.0001
North Indians [8]	346	36	20	44	14.65	0.0007
Present study	300	15	20	65	27.589	<0.00001

Table 4. Association of *Fok*I and *Taq*I SNPs with vitamin D levels

SNP	Genotypes	Vitamin D deficient women (<20ng/ml) (N) %	Vitamin D insufficient women (21-29ng/ml) (N) %	P-Value
*Fok*I	FF	(48) 60	(115) 52	<0.001*
	Ff	(3) 4	(7) 3	
	ff	(29) 36	(98) 45	
*Taq*I	TT	(22) 28	(52) 24	<0.001*
	Tt	(50) 62	(116) 52	
	tt	(8) 10	(52) 24	

*X^2 test.

Table 5. Association of *Fok*I and *Taq*I genotypes with vitamin D levels

Genotypes	Vitamin D deficient women (<20ng/ml) (N)	Vitamin D insufficient women (21-29ng/ml) (N)	P-value
FF vs TT	48 vs15	115 vs 30	0.616*
Ff vs Tt	3 vs 72	7 vs 123	0.658*
ff vs tt	51 vs 8	76 vs 52	0.000*

*X^2 test.

Each population was compared with respect to the Pakistan population and the Chi-square and *P* values calculated. The X^2 tabulated at 5% level of significance (i.e.0.05) and 1 degree of freedom.

Association of vitamin D level with *Fok*I and *Taq*I SNPs was studied in 300 individuals, who were segregated into two categories as vitamin D deficient and insufficient. Out of 300 healthy women, 80 were vitamin D deficient and 220 had insufficient levels. A significant association (*P*=<0.001) of both *Fok*I and *Taq*I SNPs with vitamin D levels was observed in present population which indicated that these SNPs are associated with vitamin D deficiency and insufficiency [Table 4]. Association of *Fok*I and *Taq*I genotypes was analyzed in Table 5 and ff vs tt genotypes were found to be significantly (*P*=0.000) associated with low levels of vitamin D as compared to FF vs TT and Ff vs Tt genotypes.

4. Discussion

Polymorphisms of the vitamin D receptor gene (*VDR*) have been widely investigated in various populations. Multiple polymorphic variations exist in the *VDR* gene, each of which could have different types of consequences. *VDR* polymorphisms can affect VDR mRNA/protein level, stability, translation efficiency and protein-protein interactions [17]. More than 25 different polymorphisms are currently known to be present at the *VDR* gene and most are in/near the regulatory areas rather than coding sequences. The most studied polymorphisms include FokI, *TaqI* and *ApaI*. A very limited data is available on *VDR* gene polymorphisms in Pakistani healthy women. Present study is the first report to describe the *VDR* gene polymorphisms (*Fok*I, *Taq*I) in healthy women of age group between 19-75years. Percentage of genotypic frequency of *VDR* gene in our population was FF=55, Ff=3, ff=42%, TT=15, Tt=65 and tt=20% (Table 1) whereas in a previous study conducted by Bid *et al* [8] it was FF=44, Ff=49, ff=7, TT=49, Tt=40 and tt=11% among healthy women of age group 20-74 years. In Emirati healthy population genotypic frequency was FF=27 , Ff= 42 and ff= 31% [22]. Garnero *et al* [18] in France determined genotypic frequency TT=3, Tt=49 and tt= 18% in healthy women. Haddad [1] evaluated genotypic frequency TT=36, Tt= 58 and tt=6% in syrian healthy population whereas in Emirati healthy population genotypic frequency was TT=38, Tt=42 and tt=20% [22]. The allelic frequency in our population is F=56 and f=44% where as in another study [8] it was reported as F=68.5 and f=3.5%. In a recent analysis by Uitterlinden *et al.*, the f allele of Fok1 occurs less frequently in Africans as compared to Caucasians and Asians (*Fok*I f: Caucasians 34%, Asians 51% , Africans 24%). In a previous study allelic frequency in Emirati population was F=48.04 and f=51.96% [22] The allelic frequency of *Taq*I polymorphism in our population was T=47.5 and t=52.5% whereas in population of North India it was T=66 and t=34% [8]. The T allele frequency percentage in Asians was reported as (Taq1 T allele: Caucasians 43% , Asians 8% , Africans 31% [23]. In syrian healthy population Haddad [1] reported allelic frequency T=65 and t=35% whereas Osman *et al.* [22] determined allelic frequency T=59.2 and t=40.8% in Emirati population. In this study, a

significant association of *Fok*I and *Taq*I SNPs with vitamin D levels was found whereas previous study conducted by Bhanushali, *et al*. [24] reported significant association of *TaqI* with vitamin D levels in indian population. Several large studies have reported ethnic variations in the occurrence of *VDR* gene polymorphisms [3,25]. The differences in our population from the other population indicated the influence of ethnicity. Most data indicate that the F allele is more effective than the f allele in trans-activation of the 1, 25(OH)2-D3 signal [17,26]. In present study (Table 5), ff and tt homozygotes women were found to have low levels of vitamin D (p=0.000) as compared to FF homozygotes and Ff heterozygotes. Low vitamin D levels leads to low bone mineral density (BMD). In Pakistan, lower BMD is quite commonly reported [27,28]. The role of VDR polymorphisms in relation to low BMD has not been investigated in Pakistani population. In other studies conducted by Harris *et al [15]* where ff homozygote women were found to had BMD (femoral neck) 7.4% lower as compared to FF homozygotes women. Another study conducted by Duman *et al*. [29] tt genotype was significantly tt ($p < 0.05$) associated with low L1–4 BMD as compared to TT genotype.

Thus, the current data signifies variation in frequency of VDR polymorphisms (*Fok*I, *Taq*I) in our population and provides a basis for future epidemiological and clinical studies. VDR gene polymorphisms might be an important factor for genetic susceptibility to vitamin D deficiency in the Pakistani population. Further studies will be needed to determine the functional consequences of different VDR alleles.

5. Conclusion

The present study demonstrated genotypic and allelic frequency of VDR gene polymorphisms (*Fok*I and *Taq*I) in healthy Pakistani women. Both *Fok*I and *Taq*I SNPs are associated with vitamin D deficiency and insufficiency. The results showed the variation in distribution of VDR polymorphisms in healthy population. Further studies are required to explain the effect of these polymorphisms on disease susceptibility in our population.

Author Disclosures

The authors have no funding or conflict of interest regarding this paper.

Acknowledgements

The authors would like to acknowledge Director, INMOL for permission to use technical facilities.

References

[1] Haddad, S. Vitamin-D receptor (VDR) gene polymorphisms (Taq-I & Apa-I) in Syrian healthy population. Meta Gene. 2: 646-650 2014.

[2] Welsh, J. E., Wietzke, J. A., Zinser, G. M., Byrne, B., Smith, K. and Narvaez, C. J. Vitamin D-3 Receptor as a Target for Breast Cancer Prevention. J. NUTR. 2003: 2425S-2433S. 2003.

[3] Uitterlinden, A. G., Fang, Y., Van Meurs, J. B, Pols, H .A. and Van Leeuwen J. P. Gene. Genetics and biology of vitamin D receptor polymorphisms. 2338 (2): 143-56. 2004.

[4] Khan, M. I., Bielecka, Z. F., Najm, M. Z., Bartnik, E., Czarnecki, J. S., Anna M. Czarnecka, A. M. et al. Vitamin D receptor gene polymorphisms in breast and renal cancer: Current state and future approaches (Review). Int. J. Oncol. 44: 349-363. 2014.

[5] Trump, D., Hershberger, P. and Bernardi, R. Anti-tumor activity of calcitriol: Pre-clinical and clinical studies. J. Steroid Biochem. Mol. Biol. 89: 519-26. 2004.

[6] Pike, J. W., Meyers, M., Watanuki, M. et al. Perspectives on mechanisms of gene regulation by 1, 25-dihydroxyvitamin D3 and its receptor. J. Steroid Biochem. Mol. Biol. 103: 389-95. 2007.

[7] Rukin, N. J. and Strange, R. C. What are the frequency, distribution and functional effects of vitamin D receptor polymorphisms as related to cancer risk?. Nutr. Rev. 65: 96-101. 2007.

[8] Bid, H. K., Mishra, D. K. and Mittal, R. D. Vitamin-D Receptor (VDR) Gene (Fok-I, Taq-I & Apa-I) Polymorphisms in Healthy Individuals from North Indian Population. APJCP. 6. 147-152. 2005.

[9] McCullough, M. L., Stevens, V. L., Diver, W. R., Feigelson,H.S., Rodriguez, c., Bostick, R. M. et al. Vitamin D pathway gene polymorphisms, diet, and risk of postmenopausal breast cancer: a nested case-control study. BCR. 9(1): 1-7. 2007.

[10] Chen, C., Liu, Q., Zhu, L., Yang, H. and Wei Lu, W. Vitamin D Receptor Gene Polymorphisms on the Risk of Tuberculosis, a Meta-Analysis of 29 Case-Control Studies. PLOS ONE. 8(12): 1-11. 2013.

[11] Mohammadi, Z., Keshtkar, A., Bakhsh, F. F., Ebrahimi, M., Amoli, M. M., Ghorbani, M., et al. Prevalence of osteoporosis and vitamin D receptor gene polymorphisms (FokI) in an Iranian general population based study (Kurdistan) (IMOS). Med. J. Islam. Repub. Iran 29: 238. 1-7. 2015.

[12] Younus, A., Faiz, M. and Yasmeen, A. Serum Vitamin D in Women with Pre and Post Menopausal Newly Diagnosed Breast Cancer in Pakistan. JFFNR. 4(12): 828-833. 2016.

[13] Holick, M. F. and Chen, T. Vitamin D deficiency: a worldwide problem with health consequences. Am. J. Clin. Nutr. 87: 1080S-6S. 2008.

[14] Masood, Z., Mahmood, Q. and Ashraf, K. T. Vitamin D Deficiency–An Emerging Public Health Problem In Pakistan. JUMDC. 1:4-9. 2010.

[15] Harris, S. S., Eccleshall, T. R., Gross, C. et al. The vitamin D receptor start codon polymorphism (Fok-1) and bone mineral density in premenopausal American Black and White women. *J. Bone Miner. Res.* 12: 1043-8. 1997.

[16] Riggs, B. L., Nguyen, T. V., Melton, L. J. et al. The contribution of vitamin D receptor gene alleles to the determination of bone mineral density in normal and osteoporotic women. J. Bone Miner. Res. 10: 991-96. 1995.

[17] Whitfield, G. K., Remus, L .S., Jurutka, P.W., Zitzer, H., Oza, A. K., Dang, H. T. et al. Functionally relevant polymorphisms in the human nuclear vitamin D receptor gene. Mol. Cell Endocrinol.177: 145-59. 2001.

[18] Garnero, P., Borel, O., Sornay-Rendu, E., et al. Vitamin D receptor gene polymorphisms do not predict bone turnover and bone mass in healthy premenopausal women. *J. Bone Miner. Res*. 10: 1283-8. 1995.

[19] Hutchinson, P. E., Osborne, J. E., Lear, J. T., Smith, A. G., Bowers, P. W., Morris, P.N. et al. Vitamin D Receptor Polymorphisms Are Associated with Altered Prognosis in Patients with Malignant Melanoma. Clin. Cancer Res. 6: 498-504. 2000.

[20] Minamitani, K., Takahashi, Y., Mingawa, M., Yasuda, T. and Niimi, H. Difference in height associated with a Translation Start Site Polymorphism in the Vitamin D Receptor Gene. Pediatr. Res. 4: 628-32. 1998.

[21] Kung, A.W., Yeung, S. S. and Lau, K. S. Vitamin D receptor gene polymorphisms and peak bone mass in southern Chinese women. Bone. 22: 389-93. 1996.

[22] Osman, E., Al Anouti, F., El ghazali, G,. Haq A., Mirgani, R., Al Safar, H. Frequency of rs731236 (Taql), rs2228570 (Fok1) of Vitamin-D Receptor (VDR) gene in Emirati healthy population. Meta Gene. 6: 49-52. 2015.

[23] Khan, M. I., Bielecka, Z. F., Najm, M. Z., Bartnik, E., Czarnecki, J. S., Czarnecka, A. M. Et Al. Vitamin D receptor gene polymorphisms in breast and renal cancer: Current state and future approaches (Review). Int. J. Oncol. 44: 349-63. 2014.

[24] Bhanushali, A. A., Lajpal, N., Kulkarni, S. S., Chavan, S. S., Bagadi, S. S. and Das, B. R. Frequency of fokI and taqI polymorphism of vitamin D receptor gene in Indian population and its association with 25-hydroxyvitamin D levels. Int. J. Hum. Genet. 15(3): 108-13. 2009.

[25] Zmuda, J. M., Cauley, J. A. and Ferell RE: Molecular epidemiology of vitamin D receptor gene variants. Epidemiol. Rev. 22: 203-217, 2000.

[26] Jurutka, P.W., Remus, L.S., Whifield, G. K., Thomson, P. D., Hsieh, J. C., Zitzer, H. *et al*. The polymorphic N terminus in human vitamin D receptor isoforms influences the transcriptional activity by modulating interaction with transcription factor IIB. Mol. Endocrinol. 14: 401-20. 2000.

[27] Akhlaque, U., Ayaz, S. B., Akhtar, N., Ahmad, N. Association of bone mineral density and body mass index in a cohort of Pakistanis: Relation to gender, menopause and ethnicity. The Egyptian Rheumatologist. 39: 39–43. 2017.

[28] Gul, M. S.,Tasneem, S., Khan, S.H.,Iftikhar, T., Gul, N.,Shah, J. Z. Decreased bone mineral density and associated risk factors in hospital visiting people of Islamabad. J Pak Med Assoc. 64(12): S11-14. 2014.

[29] Duman, B. S., Tanakol, R., Erensoy, N., Oztürk, M., Yilmazer, S. Vitamin D receptor alleles, bone mineral density and turnover in postmenopausal osteoporotic and healthy women. Med Princ Pract. 13(5): 260-6. 2004.