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Biochemistry

Association between vitamin D receptor gene polymorphisms and Hypothyroidism in Saudi population.

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ABSTRACT

Objective : To investigate three polymorphic sites *ApaI,BsmI* and *TaqI* of vitamin D receptor (VDR) gene on chromosome 12q (12–12q14) as candidate for hypothyroidism (HT) in Saudi population.

Method : A sample of 100 Saudi classified as healthy (control,N= 50 (without HT) or with HT (N= 50) was investigated the relationship of VDR gene polymorphisms at three restriction sites *Apa*I, *Bsm*I and *Taq*I to the risk of HT .Blood samples were taken and DNA was extracted from whole blood. The target part of VDR gene was isolated and amplified by the polymerase chain reaction (PCR). PCR products were digested by restriction enzymes: *ApaI, TaqI, and BsmI* and electrophoresed on agarose gel.

Result: Thyroid–stimulating hormone (TSH) levels were statistically higher in HT than controls (P-value= 2.44E-10*). The difference in Vitamin D Total (Vit.D) values between each two shows significant difference p-value =0.0207<0.05. However,we observed that were distribution of genotypes frequency of the *BsmI TaqI* and *ApaI* VDR gene polymorphisms differed significant between HT and control groups (p=.0127<0.05) for *BsmI* (p=.0150<0.05) for *TaqI* and (p=.0376<0.05) for *ApaI*.

Conclusion: The VDR gene polymorphisms at all three restriction sites *Bsm*I,TaqI and ApaI were associated with the risk of hypothyroidism in Saudi population.

KEYWORDS : Hypothyroidism (HT);Vitamin (D) receptor (VDR) ;Gene polymorphisms; Saudi population .

Introduction

Hypothyroidism is a common endocrine disorder resulting from deficiency of thyroid hormone. It usually is a primary process in which the thyroid gland is unable to produce sufficient amounts of thyroid hormone **(1)**. Several studies have shown that this disorder is quite common (10% in an elderly population) **(2,3)**. It is still a matter of controversy whether this disorder should be screened and treated **(4).**

Vitamin D is involved in biological processes such as bone metabolism, cell proliferation and differentiation **(5).** In the classical endocrine pathway, vitamin D enters the circulation attached to a D-binding protein, is first hydroxylated in the liver to 25(OH) D and then in the kidney to form the active metabolite, 1, 25 dihydroxy vitamin D (1, 25-(OH)2 D) or calcitriol **(6)**.

Vitamin D exerts its genomic action via the nuclear vitamin D receptor (VDR), which shows an extensive polymorphism. The VDR gene is located on chromosome 12q (12-12q14) and is highly polymorphic. The VDR belongs to the steroid receptor super-family and is widely expressed in many cell types, including lymphocytes, macrophages, and pancreatic –cells **(7).** Four major polymorphic sites have been described withithe VDR gene. A polymorphic FokI site in exon 2 results in an alternative transcription initiation site, leading to a protein variant with three additional amino acids at the amino terminus. Polymorphic BsmI and ApaI sites are present in intron 8, and a silent T to C substitution creates a TaqI restriction site in exon 9 **(8).**

Importantly, both vitamin D and thyroid hormone bind to similar receptors called steroid hormone receptors. A different gene in the Vitamin D receptor was shown to predispose people to autoimmune thyroid disease including Graves' disease and Hashimoto's thyroiditis. For these reasons, it is important for patients with thyroid problems to understand how the vitamin D system works **(9)**.

Recently, many studies have shown that low levels of vitamin D contribute to Graves' disease (GD) and Hashimoto thyroiditis (HT) and that combining vitamin D with anti-thyroid drugs or thyroid hormone contributes to the treatment of autoimmune thyroid disease (AITD) by suppressing the autoimmune reaction and reducing serum levels of thyroid autoantibodies **((10); (11)).** However, other authors have proposed that vitamin D deficiency does not increase the risk of AITD and is not associated with early-stage AITD **((12); (13)).** Because that the association between vitamin D levels and AITD is still controversial. This inconsistency was attributed to the environmental factors that potentially interfere with the VDR genotypes **(14).** However, at this time the research on its role in autoimmune and thyroid disease is not conclusive. The biological effect of vitamin D is thought to occur by binding to the vitamin D receptor (VDR) which belongs to the steroid receptor super family. However, *BsmI* (rs1544410), *ApaI* (rs7975232), and *TaqI* (rs731236) SNPs, located near the 3′ end of the VDR gene, are in strong linkage disequilibrium (LD) with each other. These three SNPs don't change the amino acid sequence of the encoded protein but have been shown to affect gene expression through regulation of mRNA stability **(15)**. Therefore, we have investigated three polymorphic sites *ApaI,BsmI* and *TaqI* of VDR gene on chromosome 12q (12–12q14) as candidate for HT susceptibility locus for the first time in Saudi population.

Material and Methods

Subjects

The VDR gene polymorphisms analysis study included a total of 100 Saudi volunteers. The diagnosis criteria of HT were according to the World Health Organization (WHO).Subjects were divided in two groups HT and non-HT. Total number of 50 Saudi patients (15 men and 35 women)with HT. Fifty healthy control subjects were studied (25 men and 25 women). They consisted of individuals with no history of thyroid or other autoimmune disease. This criterion depends upon two tests: Thyroid–stimulating hormone (TSH) and Vitamin D Total Test **(Vit.D)** . We recorded the clinical parameters for each HT volunteer upon diagnosis : TSH test, Vit.D test .These medical details were obtained from patient hospital files after obtaining the consent from the administration. The previous history of HT and family history of thyroid were taken from patient.The same

parameters were recorded for the control volunteer. All subjects were selected from those who routinely attended diabetic clinic, Association of Diabetic Patient Friends Taif, King Faisal Hospital (KFH), Taif, Saudi Arabia. The study was approved by the ethical committee.All participants in the study filled a questionnaire and also signed a consent form.

Study Design

Genomic DNA was extracted from whole blood samples, in bio safety cabinet, using Thermo Scientific DNA extraction kit Blood (Thermo Scientific, USA, Cat.no. K0781). The extracted DNA was stored at -20˚C for PCR amplification.

For Polymerase Chain Reaction (PCR), the reactions were prepared using Maxima Hot Start Green PCR Master Mix (2x). The primers were from Macrogen .The forward primer was (5'-CAA CCA AGA CTA CAA GTA CCG CGT CAG TGA-3') and the reverse one was (5'-GCA ACT CCT CAT GGC TGA GGT CTC-3'). For polymerase Chain Reaction (PCR), the master mix from Thermo Scientificwas used. The mixture (50µl) contained 2X reaction buffer, 4mM Mg^{+2}, 4µM deoxyribonucleoside triphosphates, 0.2µM of each primer, 0.45 U *Taq* DNA polymerase and 10-30 ng of DNA template. The total reaction volume was made up to 50µl with nuclease free water.

The PCR tubes were transferred to thermal cycle. The amplification conditions were ; an initial denaturation for 4 min at 95˚C , 30 cycles each of which consisted of (denaturation at 95 ˚C for 30 S, annealing at 60 ˚C for 1 min and an extension at 68 ˚C for 2 min), and final extension for 5 min at 72˚C and ended at hold at 4˚ C. The PCR products were verified by 1% agrose gel electrophoresis at 100 V for 30 min .Purification of Purification was done for the PCR product using Gene JET PCR Purification Kit from Thermo scientific.

VDR genotyping

Amplified PCR products (5 µl) were digested with 3000 U of each restriction enzyme from Thermo Scientific. These enzymes are *Acetobacter pasteurianus ApaI*, *Bacillus stearothermophilus BsmI* and *Thermus aquaticus YTI TaqI* .These enzymes were used to the supplier-recommended protocols by Thermo Scientific. By using the thermal cycler, the reaction was incubated in 37˚C for 1 hour which is the activation temperature of *BsmI* and *ApaI*, then the enzymes were inactivated by incubation at 65˚C for 20 min. After that, the reaction was incubated 65˚C for 1 hour which is activation temperature of *TaqI*, and then the enzymes were inactivated by incubation at 80˚C for 20 min.

Statistical analysis

All statistical analyses were performed using the MegStat® version 9.0 computer program. Descriptive data were given as mean ± standard deviation (SD).Differences among groups were tested using the t-test.Differences in distribution of the genotypes between the females and males according to HT and Non-HT were examined with the chi-square analysis. Differences in genotype frequencies were considered statistically significant for p-value < 0.05.

Results

The Main Characteristic of the study group

The volunteers in this study were classified according to Thyroid–stimulating hormone (TSH) test as two groups HT (35 Females, 15 Males) and non-HT (25 Females, 25 Males) totally 50 subjects. The clinical and biochemical parameters for the two groups HT and control sample are shown in Table 1. The Thyroid–stimulating hormone (TSH) values between HT and non- HT patients shows significant difference p-value=2.44E-10*<0.05, but non-significant difference between females and males in two groups. The vitamin D Total (Vit.D) values between each two groups females and males are significant difference p-value =0.0207<0.05 between HT and non- HT.

Table 1 Clinical and biochemical characteristics of non-HT and HT

Parameters	HT Mean ± SD	Non-HT Mean ± SD	P-value
Vitamin D Total (Vit.D) (nmol/L)	17.3±4.50	20.2±5.33	0.0207
Thyroid–stimulating hormone (TSH) (mmol/L)	12.060±8.959	2.934±1.8273	2.44E-10*

***Highly significant difference.**
Data is represented as mean ± SD
P-Value for control groups and HT.

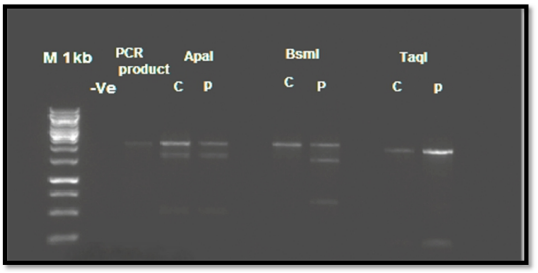

Figure 1 Digestion results of the 3 polymorphism sites. Lane 1 : 1kb DNA ladder .Lane 2 :negative control, lane 3 :PCR Products (2229 bp). *ApaI* digestion: control(2229bp, 1900bp,500bp) patient(2229bp,1900bp,500bp).*BsmI* digestion: control (2229bp), patient(2229bp,1900bp,700bp).*TaqI* digestion : control (2229bp) ,patient (2229bp,250bp).

The PCR products in our samples from Saudi volunteers, ~ 2229 bp as shown in Figure 1, were digested with the *BsmI ,TaqI and ApaI* restriction enzymes.

Statistical analysis of Genotypes Frequencies of *BsmI,TaqI* and *ApaI* Polymorphisms in study Groups at HT and Control samples

Frequencies of VDR alleles in the two groups, irrespective of whether it was in females and males, shown in Table 2 . Accordingly, the distribution of alleles frequency of the *BsmI*,TaqI and ApaI VDR polymorphisms differed significantly between hypothyrodisim and controls. The frequency of B allele was more frequent in control group compared with hypothyrodisim patients and b more in hypothyrodisim. Furthermore ,the frequency of T allele was more frequent in hypothyrodisim patients compared with control group and t more in control and frequency of A allele was more frequent in control and frequency of a allele was more frequent in hypothyrodisim. Therefore was observed significant difference between them (p= .0127<0.05) for BsmI (p=.0150<0.05) for TaqI and (p=.0376<0.05) for ApaI.

Table2 Distribution of VDR gene polymorphisms in patients with HT and non-HT

VDR polymorphisms	Case (n=50)		Controls (n=50)		P-value
	NO.	%	NO.	%	
BsmI Genotypes					
BB	17	34	24	48	0.1924
Bb	10	20	21	42	
bb	23	46	5	10	
ApaI Genotypes					0.3669
AA	11	22	14	28	
Aa	6	12	17	34	
aa	33	66	19	38	
TaqI Genotypes					0.3212
TT	21	42	18	36	
Tt	18	36	15	30	
tt	11	22	17	34	
Bsm I Alleles					*0.0127
B	44	44	69	69	
b	56	56	31	31	
ApaI Alleles					*0.0376
A	28	28	45	45	
a	72	72	55	55	

TaqI Alleles					*0.0150
T	59	59	51	51	
t	41	41	49	49	

Discussion

Vitamin D deficiency has been associated with numerous autoimmune diseases **((16); (17) ; (18); (19)) .** Not many endocrinologists realize this, but several articles published over 20 years ago showed that patients with hypothyroidism have low levels of vitamin D. This may lead to some of the bone problems related to hypothyroidism. Importantly, both vitamin D and thyroid hormone bind to similar receptors called steroid hormone receptors. Meanwhile, the vitamin D receptor (VDR) gene became a likely candidate for the common autoimmune susceptibility gene because it has been found to be associated with AITD such as Graves' disease (GD) **(20).** All these facts point to the significant association between vitamin D deficiency and AITD. A different gene in the Vitamin D receptor was shown to predispose people to autoimmune thyroid disease including Graves' disease and Hashimoto's thyroiditis **(9)** . Few studies have been conducted in order to find any significant association between the levels of vitamin D and hypothyroidism and to determine whether vitamin D deficiency involves in the pathogenesis of hypothyroidism or rather a consequence of the disease and those that yielded conflicting results. Due to these reasons, this study has been chosens to investigate the association of hypothyroidism in a sample of Saudi population with VDR *BsmI-ApaI-Taq* I polymorphisms and measured biochemical tests such as (TSH) levels and total vitamin D as a target for this study.In the present study, samples were divided into two groups hypothyroidism and non- hypothyroidism according females and males that measure biochemical tests (TSH,Vit.D). Results showed significant difference in the mean value of vitamin D between the hypothyroidism and controls was found (p=0.0207< 0.05). There was no significant difference between females and males of each two groups (p=0.3489>0.05, p=0.6142>0.05).

Therefore, Vitamin D inhibits the production of Th1 polarizing cytokine (IL-12), thereby indirectly shifting the polarization of T cells from a Th1 toward a Th2 phenotype. In the CD4+ T cell response, vitamin D directly inhibits the production of Th1 cytokines (IL2 and IFN-c), and enhances Th2 cytokine (IL-4) production **(21).** In addition, recent numerous studies have shown the relation of vitamin D and various autoimmune diseases. Vitamin D receptor (VDR) gene polymorphisms and vitamin D status are associated with different autoimmune diseases **((22); (23))**. Autoimmune thyroid diseases were more prevalent in women **(24)**. Women have been reported to have higher absolute numbers of CD4+lymphocytes and higher rates of Th1 cytokine production than men **(25)**. It is also well known that estrogen stimulates calcitriol accumulation in women **(26)**.

We examined the association of VDR gene polymorphisms at three positions (*ApaI*, *BsmI* and *TaqI*) with hypothyroidism. Our results demonstrated alleles of *BsmI* gene polymorphisms was more frequent in healthy controls compared with HT ,alleles of *TaqI* and ApaI more in HT and there were significant difference in alleles frequency between cases and controls (p= 0.0127<0.05) for BsmI (p=.0150<0.05) for TaqI and (p=0.0376<0.05)for ApaI. Therefore, allele b in *BsmI* ,allele T in TaqI and allele a in ApaI region are risk alleles in Saudi population.

This outcome matches the outcome of the largest meta-analysis which found relationship between *BsmI* and TaqI polymorphisms in VDR and HT, whereas the *ApaI* polymorphisms dose not appear to have a significant association with overall HT risk. Therefore, Three studies indicated *TaqI* polymorphism was associated with risk of HT in Croatian and Turkish population**((27); (28) ; (29))**, but four other studies from China, Japan, Italy and Serbia showed no association**((30); (31); (32); (33))**.

Furthermore, the apparent discrepancies between this study and other could be a result of the effect of ethnic differences related to the distribution of VDR polymorphisms in these population as well as interactions with other genetic or environmental factors **(34).**Therefore, the environmental factor influence levels of active vitamin D in humans are complex and a significant difference exists between vitamin D functions and VDR polymorphisms **(35).**

Some study found a higher frequency of variant allele of ApaI and TaqI, but not significantly different between patients with HT and healthy controls (P > 0.05). In most epidemiological studies, however, associations were examined between SNPs, or between the BsmI,ApaI and TaqI linkage group and the physiological parameter of interest **((36); (37))**, but there is a lack of analysis of the direct influence of allelic variation on VDR protein expression or activity. In fact, BsmI, ApaI or TaqI alleles have no effect on either the expression levels or the activity of the translated VDR protein **((38); (39)).**

Conclusion

our case control study indicated that the VDR polymorphism in *BsmI* ,*TaqI* and ApaI regions are associated with hypothyrodisim in the Saudi population .We recommend using the same methodology with larger sample size in the same population. In addition, future studies on the correlation between environmental factor such as UV, immune response, VDR SNPs and HT may also be considered.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

References:

1. American Thyroid Association Task Force on Thyroid Hormone Replacement. Jonklaas, J., Bianco, A.C., Bauer, A.J., Burman, K.D., Cappola, A.R., Celi, F.S., Cooper, D.S., Kim, B.W., Peeters, R.P., Rosenthal, M.S. and Sawka, A.M. : Thyroid, 2014, Vols. 24(12):1670-751.
2. The aging thyroid: thyroid deficiency in the Framingham study. Sawin, C.T., Castelli, W.P., Hershman, J.M., McNamara, P. and Bacharach, P. : Archives of Internal Medicine, 1985, Vols. 145: 1386–1388.
3. The incidence of thyroid disorders in the community: a twenty-year follow-up of the Wickham survey. Vanderpump, M.P.J., Tunbridge, W.N.G., French, J.M., Appleton, D., Bates, D. and Clark, F. : Clinical Endocrinology, 1995, Vols. 43:55–68.
4. Hypothyroidism: screening and subclinical disease. Weetman, A.P. : British Medical Journal, 1997, Vols. 314 :1175–1178.
5. Vitamin D and VDR gene polymorphism (FokI) in epithelial ovarian cancer in Indian population. Mohapatra, S., Saxena, A., Gandhi, G., Koner, B.C. and Ray, P.C. : J Ovarian Res, 2013, Vols. 6 (1):37.
6. Vitamin D status and cause-specific mortality: a general population study. Skaaby, T., Husemoen, L.L., Pisinger, C., Jorgensen, T., Thuesen, B.H. and M. Fenger.M. : PLoS One, 2012,Vols. 7.
7. Vitamin D receptor polymorphisms and diseases. Valdivielso, J. and Fernandez, E. : Clinica Chimica Acta, 2006, Vols. 371:1–12.
8. Genetics and biology of vitamin D receptor polymorphisms. Uitterlinden, A.G., Fang, Y., Van Meurs, J.B., Pols, H.A. and Van Leeuwen, J.P. : Gene, 2004, Vols. 338:143–156.
9. Vitamin D Deficiency and Thyroid Disease. Friedman, T. C.: www.goodhormonehealth.com/VitaminD.
10. The Study on Relationship between Serum 25-Hydroxyvitamin D3 Concentration and Hashimoto Thyroiditis. Huang, Z.L. : Master Dissertation, Jilin University, Jilin, China, 2013.
11. The Study on Relation between Vitamin D3 Level and Immune Disorder in Patients with Autoimmune Thyroid Disease. Liu, X.H. : Master Dissertation, Zhengzhou University, Zhengzhou, China, 2012.
12. Relationship of vitamin D deficiency and autoimmune thyroid diseases. Sezgin, G. and Esref, O.M. : Eur. J. Internal Med, 2011, Vols. 22:87.
13. Vitamin D deficiency is not associated with early stages of thyroid autoimmunity. Effraimidis, G., Badenhoop, K., Tijssen, J.G. and Wiersinga, W.M. : Eur J Endocrinol, 2012, Vols. 167:43–48.
14. Variation in associations between allelic variants of the vitamin D receptor gene and onset of type 1 diabetes mellitus by ambient winter ultraviolet radiation levels ameta-regression analysis. Ponsonby, A. L., Pezic, A., Ellis, J., Morley, R., Cameron, F., Carlin, J., & Dwyer, T. s.l. : American Journal of Eipdemiology, 2008, Vols. 168(4):358-365.
15. Molecular nature of the vitamin D receptor and its role in regulation of gene expression. Rev. Jurutka, P.W., Whitfield, G.K., Hsieh, J.C., Thompson, P.D., Haussler, C.A. and Haussler, M.R. : Endocr Metab Disord, 2001, Vols. 2(2):203-16.
16. Control of autoimmune diseases by the vitamin D endocrine system. Adorini, L. and Penna, G. : Nature Clinical Practice Rheumatology, 2008, Vols. 4:404.
17. Vitamin D deficiency and insufficiency in 2 independent cohorts of patients with systemic sclerosis. Vacca, A., Cormier, C., Piras, M., Mathieu, A., Kahan, A. and Allanore, Y. : Journal of Rheumatology, 2009, Vols. 36:1924.
18. Vitamin D and molecular actions on the immune system: modulation of innate and autoimmunity. Kamen, D.L. and Tangpricha, V. : Journal of Molecular Medicine (Berlin), 2010, Vols. 88:441.
19. The role of vitamin D in regulating immune responses. Toubi, E. and Shoenfeld, Y. : The Israel Medical Association Journal, 2010, Vols. 12:174.
20. Vitamin D receptor gene polymorphisms in Hashimoto's thyroiditis. Ban, Y. and Taniyama, M. : Thyroid, 2001, Vols. 11:607.
21. Vitamin D modulator of the immune system. Baeke, F., Takiishi, T., Korf, H., Gysemans, C. and Mathieu, C. : Curr Opin Pharmacol, 2010, Vols. 10(4):482–96.
22. Variation in associations between allelic variants of the vitamin D receptor gene and

onset of type 1 diabetes mellitus by ambient winter ultraviolet radiation levels: a metaregression analysis. Ponsonby, A.L., Pezic, A., Ellis, J., Morley, R., Cameron, F. and Carlin. : Am. J Epidemiol, 2008, Vols. 168(4):358–65.

23. Association of vitamin D receptor gene polymorphisms in Iranian patients with inflammatory bowel disease. Naderi, N., Farnood, A., Habibi, M., Derakhshan, F., Balaii, H., Motahari, Z., Agah, M.R., et al. : J Gastroenterol Hepatol, 2008, Vols. 23(12):1816–22.
24. Current concepts: Thyroiditis. Pearce, M.D., Farwell, M.D. and Braverman, L.E. : N Engl J Med, 2003, Vols. 348:2646-55.
25. Sex differences in autoimmune disease. Whitacre, C.C. : Nat Immunol, 2001, Vols. 2:777–780.
26. IL-12, TNF-alpha, and hormonal changes during late pregnancy and early postpartum: implications for autoimmune disease activity during these times. Elenkov, I.J., Wilder, R.L., Bakalov, V.K., Link, A.A., Dimitrov, M.A., Fisher, S., Crane, M., Kanik, K.S. and Chrousos, G.P. : J Clin Endocrinol Metab, 2001, Vols. 86:4933–4938.
27. Association of vitamin D receptor gene 3 -variants with Hashimoto's thyroiditis in the Croatian population. Stefanic, M., Papic, S., Suver, M., Glavas-Obrovac, L. and Karner, I. : Int. J. Immunogenet, 2008, Vols. 35:125–131.
28. Vitamin D receptor gene ApaI, TaqI, FokI and BsmI polymorphisms in a group of Turkish patients with Hashimoto's thyroiditis. Yazici, D., Yavuz, D., Tarcin, O., Sancak, S., Deyneli, O. and Akalin, S. : Minerva Endocrinol, 2013, Vols. 38(2):195-201.
29. Vitamin-D Receptor (VDR) Gene Polymorphisms (TaqI, FokI) in Turkish Patients with Hashimoto's Thyroiditis: Relationship to the levels of Vit-D and Cytokines. Guleryuz, B., Akin, F., Ata, M. T., Dalyanoglu, M. M. and Turgut, S. : Endocr Metab Immune Disord Drug Targets, 2016, Vols. 16(9):131–139.
30. Genetic susceptibility to autoimmune thyroid diseases in a Chinese Han population: Role of vitamin D receptor gene polymorphisms. Meng, S., He, S.T., Jiang, W.J., Xiao, L., Li, D.F., Xu, J., Shi, X.H. and Zhang, J.A. : Ann Endocrinol (Paris),2015, Vols. 76:684–689.
31. The functional polymorphisms of VDR, GC and CYP2R1 are involved in the pathogenesis of autoimmune thyroid diseases. Inoue, N., Watanabe, M., Ishido, N., Katsumata, Y., Kagawa, T., Hidaka, Y. and Iwatani, Y. : Clin Exp Immunol, 2014, Vols. 178:262–269.
32. Vitamin D receptor gene polymorphisms, haplotypes and serum 25(OH)D levels in Hashimoto's thyroiditis. Giovinazzo S., Vicchio, T., Certo, R., Alibrandi, A. et al. : Endocrine, 2016, Vols. 55(2):599-606.
33. Association between FokI, ApaI and TaqI RFLP polymorphisms in VDR gene and Hashimoto's thyroiditis: preliminary data from female patients in Serbia. Djurovic, J., Stojkovic, O., Ozdemir, O., Silan, F., Akurut, C., Todorovic, J., Savic, K. and Stamenkovic, G. : Int J Immunogenet, 2015, Vols. 42(3):190-4.
34. Association between vitamin D receptor gene polymorphisms and type 1 diabetes mellitus in Iranian population. Mohammadnejad, Z., Ghanbari, M., Ganjali, R., Afshari, J. T., Heydarpour, M., Taghavi, S. M., ... & Rafatpanah, H. s.l. : Molecular biology reports, 2012, Vols. 39(2):831-837.
35. Vitamin D receptor gene polymorphisms in type 1 diabetes mellitus: a new pattern from Khorasan province, Islamic Republic of Iran/Polymorphismes du gène du récepteur de la vitamine D et diabete de typeI:un nouveau modele dans la province de khorasan. Bonakdaran, S., Abbaszadegan, M. R., Dadkhah, E., & Khajeh-Dalouie, M. s.l. : Estern Mediterranean Health, 2012, Vol. 18(6)614.
36. Allelic variations of the vitamin D receptor (VDR) gene are associated with increased risk of coronary artery disease in type 2 diabetics: the DIABHYCAR prospective study. Ferrarezi, D.A., Bellili-Munoz, N., Dubois-Laforgue, D., Cheurfa, N., Lamri, A., Reis, A.F. et al. : Diabetes & Metabolism, 2013, Vols. 39:263–270.
37. The association of polymorphisms of the vitamin D receptor gene with psoriasis in the Han population of northeastern China. Zhou, X., Xu, L.D. and Li, Y.Z. : Journal of Dermatological Science, 2014, Vols. 73:63.
38. Functionally relevant polymorphisms in the human nuclear vitamin D receptor gene. Whitfield, G.K., Remus, L.S., Jurutka, P.W., Zitzer, H., Oza, A.K., Dang, H.T. et al. : Molecular and Cellular Endocrinology, 2001, Vols. 177:145.
39. Vitamin D. Dusso, A.S., Brown, A.J. and Slatopolsky, E. : Am J Physiol Renal Physiol, 2005, Vols. 289:8–28.