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Original article

Association between Vitamin D Receptor methylation with vitamin D, parathyroid hormone levels and lipid profile in normal and obese Saudi Females

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Abstract. Obesity has been encountered as one of the most common disorder with major public health implications. Various epidemiologic, genetic and metabolic data have shown significant role of vitamin D in obesity. The purpose of this study was to determine the connotation between VDR methylation at three sites and various variables such as weight, height, BMI, waist circumference, lipids profile (TG, HDL-C, LDL-C and total cholesterol), vitamin D, and PTH in normal and obese Saudi females. For this purpose; 100 Saudi females (31 normal weight with BMI<25 and 69 obese with BMI>30) with no chronic diagnosed diseases aged from 18 to 60 years were evaluated. Spearman rank correlation coefficient (R) with graphic representations using linear regression has been used to find the correlation between various variables. In studying the VDR methylation correlations with different variables, the results showed that; methylation of VDR at site 1 showed positive non-significant correlation with weight, BMI, serum TG and LDL levels in addition to serum vitamin D level. On the other hand, there was negative correlation with TC, HDL and PTH (parathyroid hormone). These results indicated that; methylation at site 1 could decrease PTH, HDL & TC levels. Furthermore, methylation of VDR at both site 2 and site 3 showed positive non-significant correlations with TC, TG, LDL levels in addition to serum levels of vitamin D and PTH. While it showed a negative correlation with only HDL level. Methylation of VDR at site 2 and 3 is related with obesity, it was significantly correlated with weight, BMI and waist circumference.

Key words: VDR (vitamin D receptor); BMI; CpG methylation; PTH.

Introduction

In clinical practice obesity has been encountered as very common disorder with major public health implications. It is a complex chronic metabolic disease which is defined by BMI of greater than 30 Kg/m2 (1). It is described by deposition of excessive body fat as a result of metabolic imbalance between energy intake and expenditure (2) It is a global health issue affecting more than 500 million people worldwide. It's pervasiveness varies in different zones of the world and in Saudi Arabia, almost 34% of adults has been reported to be obese (3). Its pathogenesis is multifaceted and is affected by several factors such as environmental and genetic but yet are not fully clarified (4).

Vitamin D is a fat-soluble vitamin and it is obtained from both exposure to sunlight and through dietary intake. 7-hydrocholesterol present in the skin is converted into inactive vitamin D precursors by the ultraviolet light. This precursor is then converted

into active form through two hydroxylation reactions occurring in the liver and kidney. It plays vital role in immunity and bone health (5). Its deficiency is very common throughout the world including Saudi Arabia (6) and various findings have revealed that serum level of 25 (OH)-vitamin D is associated with obesity and its complications (7).

The active vitamin-D form, wields its effect through interaction with vitamin-D receptors (VDR; a member of the nuclear receptor superfamily) and in order to permit specific DNA binding these VDR forms homodimers or heterodimers with the retinoid X receptor (RXR, NR2B). The binding of VDR-RXR complex with 1α,25(OH)2D3 is followed by the attachment of this complex to vitamin D responsive elements, which then initiate transcription in the promoter of target genes (8, 9).

In addition, VDR are highly expressed in preadipocytes from obese subjects. Studies have shown that the active form of vitamin D persuades both adipogenesis and VDR expression (10, 11).

This objective of this study was to find the connotation between VDR methylation at three sites and the variables such as weight, height, BMI, waist circumference, lipids profile (TG, HDL-C, and TC) in addition to LDL-C, Vitamin D, and PTH in normal and obese female Saudi subjects.

Materials and Methods

Subjects

Target females were 100 (18 to 60 years) randomly selected Saudi adults females (31 normal weight with BMI<25 and 69 obese with BMI>30). They have no chronic diseases, and all of them were attending fitness Clubs in different areas (North, East, West and Central area) within Riyadh city, Saudi Arabia.

Ethics Approval and Consent to Participate

The study protocol was approved by King Saud University, Saudi Arabia (Reference #: E-19-4028) and study is in concurrence with the Policy of Research Centre and within the ethical principle of the Declaration of Helsinki. The aim of this study was elucidated and written consent was taken from the all respondents.

Tools of the study

The study tools included an interview questionnaire, anthropometric measurements, body composition analysis, and blood biochemical tests.

Anthropometric measurements

Following variables were selected for anthropometric evaluation: weight (kg), and height (cm) for calculating BMI, and waist circumference (cm).

Four physical measurements were performed as follows:

Weight: Weight was measured using an electronic balance and the weight was recorded in kg to the nearest 0.1 kg.

Height: The height was measured using an enclosed ruler connected to the electronic scale above and the height of the cm was measured to the nearest 0.1 cm.

Body mass index: It was calculated using the below mentioned equation: BMI = Weight in kg / height cm in square meters.

Waist circumference: It was measured in meters and noted by centimeters to the nearest 0.1 cm.

BMI	Classification
< 18.5	underweight
18.5–24.9	normal weight
25.0–29.9	overweight
30.0–34.9	class I obesity
35.0–39.9	class II obesity
≥ 40.0	class III obesity

Biochemical assessment

Blood samples (4mL) were withdrawn from all subjects by a nurse after an overnight fast (>12 h) and transferred immediately into two non-heparinized tubes. Serum samples were stored at −80°C until required for analysis. Parameters such as fasting serum levels of lipids profile (TG, HDL-C, and TC) were measured using UDICHEM-300 chemistry Analyzer

(by REF UI59L, REF UI41HD, and REF UI 24). While LDL-C was estimated mathematically, Vitamin D, and PTH were measured using the Cobas e 602 analyzer (Roche Diagnostics, Indianapolis, IN, USA).

DNA methylation analysis

Gentra Puregene Blood Kit (Qiagen, Valencia, CA) was used for purifying DNA from whole blood samples. DNA methylation levels at CpG sites were measured using pyrosequencing (Pyromark Q24, QIAGEN-Biotage). Combined with the NaBis DNA treatment, pyrosequencing is a quantitative real-time sequencing technology that allows to measure DNA methylation levels (%) at a single cytosine (CpG) of a given genomic region. The NaBis treatment of DNA (EpiTect Bisulfite Kit, Qiagen) specifically converts unmethylated cytosines into uracil, while the methylated cytosines are protected from this transition, creating a cytosine/thymine polymorphism. Once treated, NaBis-DNA is amplified (Pyromark PCR kit, Qiagen), and the cytosine and thymine alleles are quantified by pyrosequencing (12, 13). Genotyping of the VDR single nucleotide polymorphism (SNP) was performed in the blood using pyrosequencing as reported before.

Statistical analysis

Quantitative data has been presented in terms of mean and standard division (SD).Comparison between differences in groups was done using Independent Samples T-Test for two parametric groups and Mann-Whitney Test was used to make comparison between two nonparametric groups.Correlation between various variables was done using Spearman rank correlation coefficient (R) with graphic representations using linera regression. A probability value (p value) less than or equal to (0.05) was considered as significant. All statistical analysis was done using statistical software SPSS statistical program version (16.0). Graphs were done using SPSS statistical program version (16.0) and Microsoft Excel program version 2016.

Results

The results collected from 100 randomly selected Saudi females with no diagnosed chronic diseases, were categorized in two groups according to BMI where 31 subjects were normal weight and 69 subjects were

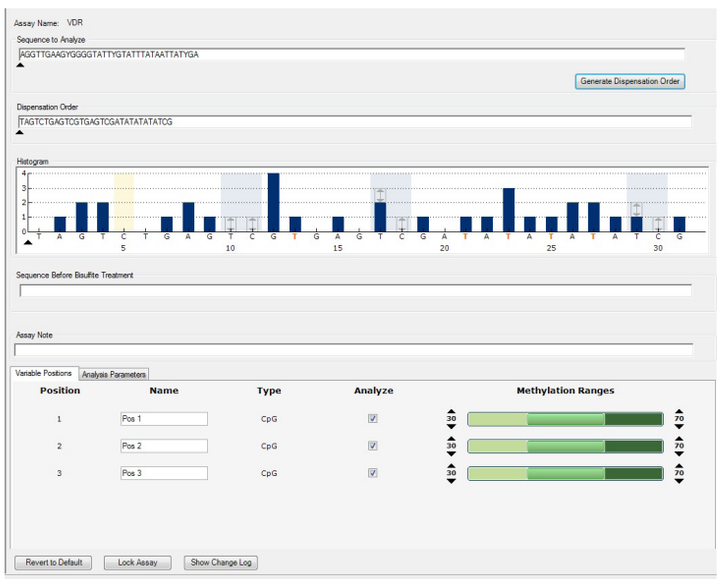

Fig. 1: DNA methylation levels at CpG sites.

Table 1. VDR Methylation

Parameters	Groups	N	Mean ± S.D.	P value
Site1 [a]	Normal	31	4.94 ± 2.53	0.728
	Obesity	69	5.22 ± 3.08	
Site2 [a]	Normal	31	7.26 ± 3.02	0.030
	Obesity	69	8.90 ± 3.97	
Site3 [a]	Normal	31	18.71 ± 6.80	0.013
	Obesity	69	23.26 ± 8.94	

[a] *Comparing between groups using Mann-Whitney Test (Nonparametric Data)*

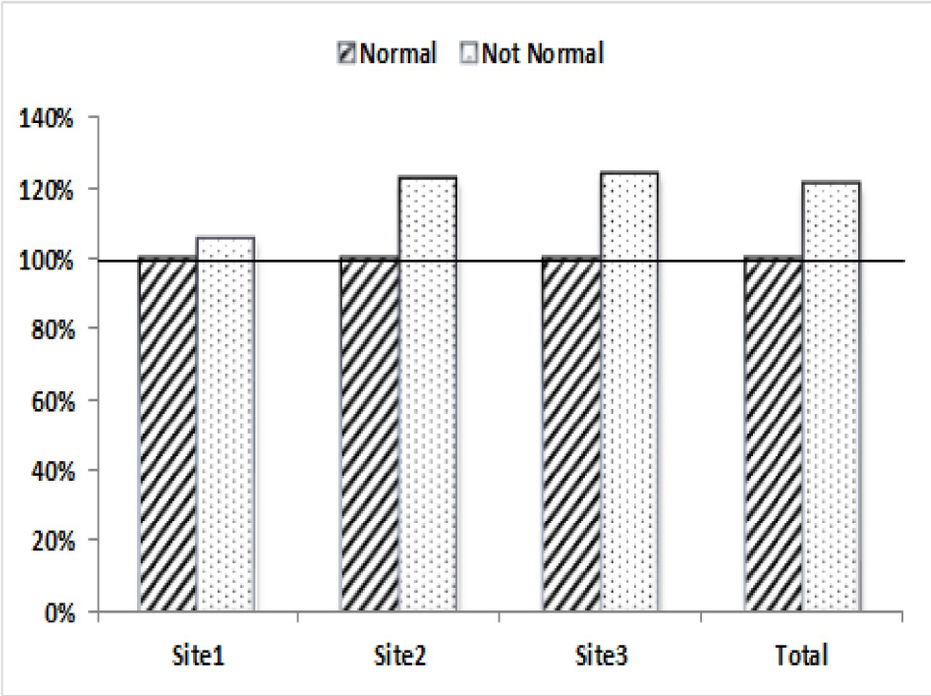

Fig. 2: Percentage change of all parameters in Obesity group compared to Normal group.

obese. DNA methylation levels at CpG were done in three sites as showed Figure 1. The most abundant methylation was placed at site 3 in obese group 23.26 ± 8.94 while methylation at site 1 in normal group showed the lowest value (Table 1 and Figure 2).

In studying the VDR Methylation Correlations with different variables, the results showed that; methylation of VDR at site 1 showed positive non-significant correlation with weight, BMI, serum TG (triglycerides) and LDL (low density lipoprotein) levels in addition to serum vitamin D level. On the other hand, there was negative correlation with TC (total cholesterol), HDL (high density lipoprotein) and PTH (parathyroid hormone). These results indicated that; methylation at site 1 could decrease PTH, HDL and TC levels (Table 2).

Furthermore, methylation of VDR at both site 2 and site 3 showed positive non-significant correlations with TC, TG, LDL levels in addition to serum levels of vitamin D and PTH. While it showed a negative correlation with only HDL level (Table 2). In addition, the results indicated that; at site 2 of VDR methylation

Table 2. VDR Methylation Correlations

Parameters	R (Spearman Correlation)	Sig.	
Site1 with Weight	0.099	0.329	P[a]
Site1 with BMI	0.095	0.346	P[a]
Site1 with Waist	0.127	0.208	P[a]
Site1 with TC	-0.082	0.419	N[b]
Site1 with TG	0.058	0.567	P[a]
Site1 with HDL	-0.103	0.309	N[b]
Site1 with LDL	0.000	1.000	P[a]
Site1 with VD	0.009	0.939	P[a]
Site1 with PTH	-0.040	0.698	N[b]
Site2 with Weight	0.273**	0.006	P[a]
Site2 with BMI	0.258**	0.010	P[a]
Site2 with Waist	0.283**	0.004	P[a]
Site2 with TC	0.044	0.662	P[a]
Site2 with TG	0.099	0.328	P[a]
Site2 with HDL	-0.124	0.223	N[b]
Site2 with LDL	0.008	0.939	P[a]
Site2 with VD	0.043	0.695	P[a]
Site2 with PTH	0.118	0.250	P[a]
Site3 with Weight	0.287**	0.004	P[a]
Site3 with BMI	0.280**	0.005	P[a]
Site3 with Waist	0.307**	0.002	P[a]
Site3 with TC	0.143	0.156	P[a]
Site3 with TG	0.169	0.092	P[a]
Site3 with HDL	-0.178	0.078	N[b]
Site3 with LDL	0.138	0.171	P[a]
Site3 with VD	0.026	0.812	P[a]
Site3 with PTH	0.071	0.491	P[a]

** *Correlation is significant at the 0.01 level.*
a Positive Correlation.
b Negative Correlation.

there was a significant positive correlation with three variables (BMI, weight and WC). These correlations were illustrated in figures 3.

Similarly at site 3 of VDR methylation there was a significant positive correlation with three variables (BMI, weight and waist circumference). These correlations were illustrated in figure 4. These results prove that methylation at sites 2 and 3 occurred in obese subjects rather than normal subjects.

Discussion

Prevalence of obesity has become worldwide issue. Various epidemiologic, genetic and metabolic data have shown significant role of vitamin D in obesity and a connotation has been found between low vitamin D level and obesity (14-17). Less exposure to sun light among obese people due to low physical activities

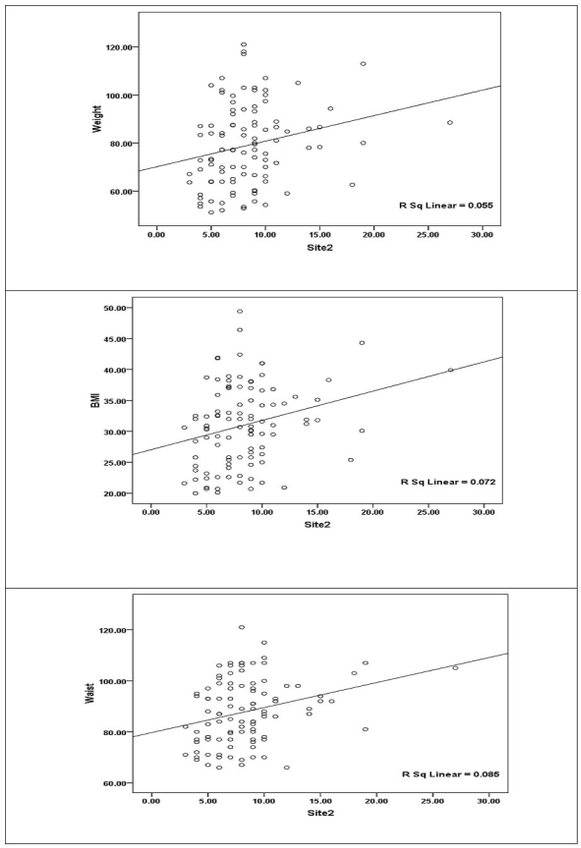

Fig.3: Correlation between Site2 and Weight, BMI and Waist with best fit line curve (positive correlation)

could explain that relation (18). Furthermore, another study have shown that in obese people the decreased vitamin D levels are as a consequence of sequestration of this vitamin by adipose tissue (19).

Vitamin D receptor (VDR) mediates the action of vitamin D. Vitamin D3 which is biologically inert get activated upon binding to the VDR. The VDR gene encrypts a ligand-activated transcription factor mediating multiple actions of vitamin D comprising cell growth, cell differentiation, calcium homeostasis, modulation of the immune response and activation of monocytemacrophages (10). Apart from this it has also been demonstrated in a wide-ranging tumors and malignant cell types, in the inhibition of cancer cell growth (20-22). Earlier, numerous studies were performed to find the connotation of obesity and different gene polymorphisms, and one of these genes intricated in these studies was VDR. In disease state this vitamin parades antiproliferative and immune-modulatory effects through its receptor (23). A significant correlation between VDR methylation and obesity has been observed in this study which is in covenant with the previous studies (24, 25). The results of this study indicated that; at sites 2 and 3 of VDR methylation there was a significant positive correlation with three variables (weight, BMI and WC).

Previous studies have evaluated the evidence of the correlation between VDR and lipids profile. Wang et al., (2009) (25) suggested that dearth of the functional VDR may lead to an upsurge in serum HDL-C and cholesterol. In addition, this can be partially elucidated by vicissitudes in the expression of genes associated with cholesterol metabolism. However, gender and diet also could affect serum lipid concentrations. Our results agreed with these results; in this study the

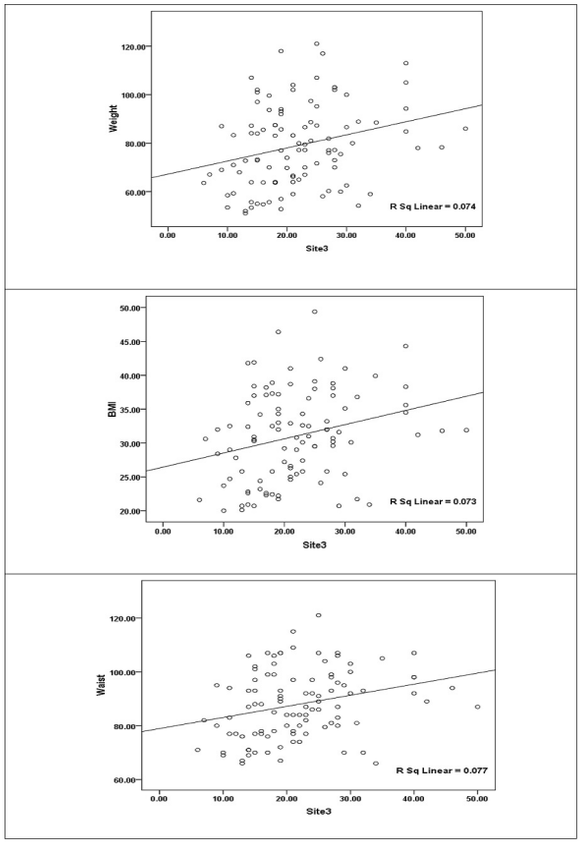

Fig.4: Correlation between Site3 and Weight, BMI and Waist with best fit line curve (positive correlation)

inverse correlation between VDR methylation and HDL supports the previous findings.

Another previous study (26) concluded that; serum 25(OH)D was positively allied with HDL-C causing in a favorable LDL-C (or total cholesterol) to HDL-C ratio. A negative relation between triglycerides and serum 25(OH)D has also been reported but these results are not uniform with our results.

VDRs are found in various tissues, like muscle tissue, brain, cardiomyocytes, immune cells, breast cells etc (27,28). In smooth muscle and vascular endothelial cells; the expression of VDR converts 25-OH vitamin D into dihydroxycholecalciferol. Dihydroxycholecalciferol is the active metabolic form of vitamin D which is regulated by PTH. Insufficient vitamin D leads to the excess production of PTH and, consequently, secondary hyperparathyroidism, cardiomyocyte hypertrophy and vascular remodeling – ventricular hypertrophy (29). In this study; the results suggested a negative connotation between PTH and VDR methylation at site 1 and a non-significant positive connotation between PTH and methylation at both sites 2 and 3.

Conclusion

It can be concluded that methylation of VDR at three sites did not significantly affected either serum levels of vitamin D or PTH and lipid profile (TC, TG, LDL, HDL). But methylation at site 2 and 3 was significantly linked with weight, BMI and WC and was related with obesity.

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References

1. WHO. Obesity: preventing and managing the global epidemic. Report of a WHO Consultation (WHO Technical Report Series 894).2000; pp: 252.
2. Nan C, Guo B, Warner C, Fowler T, Barrett T, Boomsma D, Nelson T, Whitfield K, Beunen G, Thomis M, Maes H, Derom C, Ordon J, Deeks J, Zeegers M. Heritability of body mass index in pre-adolescence, young adulthood and late adulthood. Eur J Epidemiol 2012; 27:247–253.
3. Habib SS. Body mass index and body fat percentage in assessment of obesity prevalence in Saudi adults. Biomed Environ Sci 2013; 26: 94–9.
4. Lazarevich I, Irigoyen-Camacho ME, Velazquez-Alva Mdel C. Obesity, eating behavior and mental health among university students in Mexico City. Nutr Hosp 2013; 28: 1892–9.
5. Parikh SJ, Edelman M, Uwaifo GI, Freedman RJ, Semega-Janneh M, Reynolds J, Yanovski JA. The relationship between obesity and serum 1, 25-dihydroxy vitamin D concentrations in healthy adults. J Clin Endocrinol Metab 2004; 89: 1196–9.
6. Al-Hazmi AS. Relation of vitamin D with bone mineral density and bone turnover markers in healthy Saudi men. International Journal of Advanced Research 2016; 4: 205-11.
7. Vimeswaran KS, Berry DJ, Lu C, et al. Causal relationship between obesity and vitamin D status: Bi-directional Mendelian randomization analysis of multiple cohorts. PLoS Med 2013; 10: 1549–676.
8. Carlberg C, Seuter S, Heikkinen S. The first genome wide view of vitamin D receptor locations and their mechanistic implications, Anticancer Research 2012; 32:271–282.
9. Pike JW, Meyer MB, Bishop KA. Regulation of target gene expression by the vitamin D receptor—an update on mechanisms. Reviews in Endocrine and Metabolic Disorders 2012; 13: 45–55.
10. Wong KE, Kong J, Zhang W, Szeto FL, Ye H, Deb DK, Brady MJ, Li YC. Targeted expression of human vitamin D receptor in adipocytes decreases energy expenditure and induces obesity in mice. J Biol Chem 2011; 286: 33804–33810.
11. Ochs-Balcom H, Chennamaneni R, Millen A, Shields P, Marian C. Vitamin D receptor gene polymorphisms are associated with adiposity phenotypes. Am J Clin Nutr 2011; 93: 5–10.
12. Dejeux E, El Abdalaoui H, Gut IG, Tost J. Identification and Quantification of Differentially Methylated Loci by the Pyrosequencing™ Technology. DNA Methylation;Methods in Molecular Biology 2009; 507:189–205.
13. Houde AA, Légaré C, Biron S, Lescelleur O, Biertho L, Marceau S, Tchernof A, Vohl AC, Hivert MF, Bouchard L. Leptin and adiponectin DNA methylation levels in adipose tissues and blood cells are associated with BMI, waist girth and LDL-cholesterol levels in severely obese men and women. BMC medical genetics 2015; 16: 29.
14. Dubois L, Ohm Kyvik K, Girard M, Tatone-Tokuda F, Perusse D, Hjelmborg J, Skytthe A, Rasmussen F, Wright MJ, Lichtenstein P, Martin NG. Genetic and environmental contributions to weight, height, and BMI from birth to 19 years of age: an international study of over 12,000 twin pairs. PLoS One 2012; 7:e30153.
15. Yao Y, He L, Duan Y, Liang W, Nie Z, Jin Y, Wu X, Fang Y. A meta-analysis of the relationship between vitamin D deficiency and obesity. Int J Clin Exp Med 2015; 8: 14977–84.
16. Lee S, Clark S, Gill R, Christakos S. 1,25-Dihydroxyvitamin D3 and pancreatic b-cell function: vitamin D receptors, gene expression, and insulin secretion. Endocrinology 1994; 134: 1602–10.
17. Afzal S, Brondum-Jacobsen P, Bojesen S, Nordestgaard B. Vitamin D concentration obesity and risk of diabetes: a Mendelian randomization study. Lancet Diabetes Endocrinology 2014; 2: 298–306.
18. Kull M, Kallikorm R, Lember M. Body mass index determines sunbathing habits: Implications on vitamin D levels. Intern Med J 2009; 39: 256–8.
19. Blum M, Dolnikowski G, Seyoum E, et al. Vitamin D_3 in fat tissue. Endocrinology 2008; 33: 90–4.
20. Shabahang M, Buras RR, Davoodi F, Schumaker LM, Nauta RJ, Evans SRT. 1, 25-Dihydroxyvitamin D3 receptor as a marker of human colon carcinoma cell line differentiation and growth inhibition. Cancer Research 1993; 53: 3712–3718.
21. Buras RR, Schumaker LM, Davoodi F, Brenner RV, Shabahang M, Nauta RJ, Evans SR. Vitamin D receptors in breast cancer cells. Breast Cancer Research and Treatment 1994: 31:191–202.
22. Anderson MG, Nakane M, Ruan X, Kroeger PE, Wu-Wong JR. Expression of VDR and CYP24A1 mRNA in human tumors. Cancer Chemotherapy and Pharmacology 2006; 57: 234–240.

23. Ye WZ, Reis AF, Dubois-Laforgue D, Bellanné-Chantelot C, Timsit J, Velho G. Vitamin D receptor gene polymorphisms are associated with obesity in type 2 diabetic subjects with early age of onset. Eur J Endocrinol 2001; 145: 181–6.
24. Ács O, Péterfia B, Hollósi P, Luczay A, Török D, Szabó A: Methylation Status of CYP27B1 and IGF2 Correlate to BMI SDS in Children with Obesity. Obes Facts 2017; 10:353–362.
25. Wang R, Keisala T, Solakivi T, Minasyana A, Kalueff AV, Tuohimaa P. Serum cholesterol and expression of ApoAI, LXRβ and SREBP2 in vitamin D receptor knock-out mice. Journal of Steroid Biochemistry and Molecular Biology 2009; 113: 222–226.
26. Jorde R, Grimnes G. Vitamin D and metabolic health with special reference to the effect of vitamin D on serum lipids. Prog Lipid Res 2011; 50:303–12.
27. Holick MF. Vitamin D status: measurement, interpretation and clinical application. Ann Epidemiol 2009; 19: 73–8.
28. DeLuca HF. Overview of general physiologic features and functions of vitamin D. Am J Clin Nutr 2004; 80: 1689-96.
29. Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, Lanier K, Benjamin EJ, D'Agostino RB, Wolf M, S Vasan R. Vitamin D deficiency and risk of cardiovascular disease. Circulation 2008; 117:503–11.

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