

Vitamin D receptor gene polymorphisms as a risk factor for obesity in Saudi men

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Abstract

Background: The prevalence of obesity and vitamin D deficiency in Saudi Arabia has increased recently. Decreased physical activity might play a role in obesity. Previous studies showed an association between low vitamin D level and its receptor polymorphism with obesity development.

Objective: To determine association of low vitamin D level and its receptor polymorphism with obesity in Saudi men.

Methods: This case control study was carried out from March 2016 through March 2017. Three hundred Saudi male students (from applied medical sciences in Taif University, Taif, Saudi Arabia) were classified according to BMI into lean, overweight and obese groups. For each individual, blood glucose, cholesterol, HDL-C, LDL-C, insulin and 25-(OH) vitamin D were measured. In addition, Apal, BsmI and TaqI genotypes were performed for each individual from March 2016 through March 2017, through computer-based search of the following databases: PubMed, Web of Science (Thomson Reuters™). The references of the original literature and the related articles were also searched, for potential complementary studies. Data were analyzed by SPSS version 16, using Spearman's rho and ANOVA tests.

Results: There was significant negative association between 25-(OH) vitamin D level and obesity ($p < 0.01$). Genotyping study showed that both bb of BsmI and tt of TaqI genotypes were higher in the obese group compared with lean group ($p < 0.05$). Moreover, bb genotype has higher BMI and HOMA-IR than both BB and Bb; and tt genotype also has higher BMI and HOMA-IR than TT and Tt genotypes ($p < 0.05$).

Conclusion: Low vitamin D level and VDR BsmI and TaqI genotypes may be a risk factor of obesity.

Keywords: Vitamin D receptor, Obesity, Genetic polymorphism

1. Introduction

Obesity is a complex chronic metabolic disease defined as a body mass index (BMI) of ≥ 30 Kg/m² (1). It is characterized by accumulation of excessive body fat resulting from metabolic imbalance between energy intake and expenditure (2). Obesity has become a global health problem that is associated with the development of type 2 diabetes mellitus, hypertension and cardiovascular diseases (3). It affects over 500 million people worldwide, and in Saudi Arabia its prevalence has elevated to affect 34% of adults (4). Besides environmental factors, genetic differences play an important role in the development of obesity (5). Vitamin D is a fat-soluble vitamin, obtained either from exposure to sunlight or through dietary intake (2). The ultraviolet light converts 7-hydrocholesterol present in the skin into inactive vitamin D precursors. This precursor converts into active form through two hydroxylation reactions occurring in the liver and kidney. It is responsible for bone health, immunity, and regulates

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growth and differentiation of various cell types (6). The deficiency of vitamin D is highly distributed worldwide. In Saudi Arabia, which is a sunny area, previous studies showed that the vitamin D deficiency and insufficiency reached up to 90% of all study samples (7). Several studies suggest that serum level of 25 (OH)-vitamin D is associated with obesity and its complications (8). Previous studies found that low vitamin D level, increases obesity risk in women because the women in Saudi Arabia are not exposed to sunshine (9). Active vitamin-D form, exerts its effect through interaction with vitamin-D receptors (VDR). These receptors are highly expressed in preadipocytes from obese subjects (10). Recent study found that the active form of vitamin D induces both VDR expression and adipogenesis (11). Moreover, a study done on transgenic mice that over-express human VDR in adipocytes, showed marked decreases in energy expenditure and development of obesity (12). Thus, this study was aimed to determine the association between the variables “low vitamin D level and its receptor polymorphisms Apal, BsmI and TaqI” and the development of obesity in Saudi subjects.

2. Material and Methods

2.1. Setting and subjects

The present research was a case control study. Three hundred Saudi male students were classified according to BMI into lean, overweight and obese groups. For each individual, blood glucose, cholesterol, HDL-C, LDL-C, insulin and 25-(OH) vitamin D were measured. In addition, Apal, BsmI and TaqI genotypes were performed for each individual from March 2016 through March 2017 through computer-based searches of the following databases: PubMed, Web of Science (Thomson ReutersTM). The references of the original literature and the related articles were also searched for potential complementary studies. We confirmed that no subject included in this study suffered from any chronic diseases such as diabetes mellitus or hypertension and no medications or vitamin supplementation were taken. After an overnight fast, blood samples were obtained in ethylene diamine tetra acetic acid (EDTA) in plain tubes, then separated immediately.

2.2. Ethics of research

Approval for the study was obtained from the Scientific Research Ethics Committee of Taif University with the reference number 142350.

2.3. Data collection

2.3.1. Biochemical markers:

The plasma was used for biochemical markers include glucose, triglyceride, total cholesterol, HDL-C, LDL-C, insulin and 25-(OH) vitamin D. Triglyceride, cholesterol, HDL-C and LDL-C were measured in a dimension autoanalyzer (Dade Behring Inc). Insulin and 25-(OH) vitamin D were measured by using ELISA technique (13). Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated as (fasting insulin (uIU/mL) x fasting glucose (mmol/L))/22.5.

2.3.2. Genotyping:

Blood samples collected in EDTA tubes where the DNA was extracted from peripheral blood leukocytes using the Thermo SCIENTIFIC DNA isolation kit (Thermo SCIENTIFIC) (14). Genomic DNA was amplified and analyzed for VDR genotype by polymerase chain reaction (PCR) and restriction fragment length polymorphisms (RFLP) for both TaqI and Apal genotypes using forward and reverse primers showed in Table 1. The PCR mix contained 5 µL of each primer (10 pmol), 5 µL buffer, 1.5 µL MgCl₂ (50 mM), 5 µL template DNA (50-100 ng), 5 µL dNTPs (2 mmol/L), Taq polymerase (MBI) 2 µL, H₂O 26.5 µL. The DNA template was denatured at 95 °C for 2 minutes. A total of 40 cycles of PCR were performed, consisting of denaturation step for 45 seconds at 94 °C, an annealing step for 45 seconds at optimum temperature (67 °C for Apal/TaqI and 60°C for BsmI), and an extension reaction for 1 minute at 72 °C. A final extension step at 72 °C for 2 minutes was added after the last PCR cycle. After amplification, the PCR products were digested by incubation with restriction enzymes. For Apal polymorphism, the amplicon incubated with Apal enzyme in 37 °C for 5 minutes to get its three genotypes on 1.5% agarose gel designated AA, Aa and aa. Incubation of amplicon with TaqI at 65 °C for 4 hours produced TT, Tt and tt on 2.5% agarose gel. BsmI genotypes were produced after incubation of amplicon with BsmI enzyme at 65 °C for 15 minutes, then applied on 2% agarose gel (15). The size of each genotype's Apal, TaqI and BsmI were shown in Table 1.

2.4. Statistical analysis

SPSS software version 16 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The correlations were tested using Spearman's rho. ANOVA was used in comparisons performance. Both comparisons and correlations were considered statistically significant when $p < 0.05$.

Table 1. Forward and reverse primers.

SNP	Sequences	Restriction products (bp)
Apal	Forward 5'-CAGAGCATGGACAGGGAGCAAG-3' Reverse 5'-GCAACTCCTCATGGCTGAGGTCTCA-3'	Allele AA: 740 Allele Aa: Allele aa: 515+225
TaqI	Forward 5'-CAGAGCATGGACAGGGAGCAAG-3' Reverse 5'-GCAACTCCTCATGGCTGAGGTCTCA-3'	Allele TT: 495+245 Allele Tt: 495+290+ 245+205 Allele tt: 290+245+205
BsmI	Forward 5'-AGTGTGCAGGCGATTCGTAG-3' Reverse 5'-ATAGGCAGAACCATCTCTCAG-3'	Allele BB: 360 Allele Bb: 360+191+169 Allele bb: 191+169

3. Results

This study consists of 300 male students of applied medical sciences in Taif University, classified according to BMI into lean, overweight and obese groups. Table 2 represents a comparison of anthropometric and biochemical parameters between three groups by using ANOVA. It showed significant statistical difference in insulin [lean (54.22±14.55), overweight (74.32±29.61) and obese (109.44±39.91)] with higher level in the obese group compared with the lean group (p=0.04). Moreover, HOMA-IR showed a significant statistical difference between all three groups with higher value in the obese group (7.89±3.77) compared with the lean (1.58±0.51) group (p=0.01). Our parameter of interest, 25-(OH) vitamin D showed significant statistical differences between three groups with lower level in the obese group (12.7±6.1) compared with the lean (29.5±8.7) group (p=0.00). Table 3 represents the comparison of Apal, TaqI and BsmI genotypes between lean, overweight and obese groups. The Apal genotypes did not show significant difference between all three groups, but TaqI genotypes showed that tt genotype had more frequency in the obese group compared with the lean group (p=0.021). Moreover, t allele is highly present in the obese group compared with the lean group (p=0.041). According to BsmI genotypes, the bb genotype showed higher frequency in the obese group compared with the lean group (p=0.042). In addition, the b allele showed high frequency in the obese group compared with the lean group (p=0.044). Table 4 represents the comparison between BMI, HOMA-IR and 25-(OH) vitamin D of different genotypes in the obese group, it showed that tt genotype of TaqI has higher BMI and HOMA-IR compared with TT and Tt genotypes (p=0.048). In BsmI genotypes, the bb genotype has higher values in BMI and HOMA-IR compared with both BB and Bb genotypes (p<0.05). Finally, Apal genotypes did not show any significant statistical differences in any of the three parameters.

Table 2. Comparison between biochemical parameters of lean, overweight and obese groups using ANOVA (Mean±SD)

Variable	Lean; n=100	Overweight; n=100	Obese; n=100	p-value
Age (years)	27.25±4.30	27.25±4	27.25±4.37	0.855
Height (m)	1.6±0.23	1.7±0,07	1.7±0.07	0.0726
Weight (kg)	69.9±6.4	83.8±6.6	104±17.7	0.001*
BMI (kg/m ²)	23.4±1.3	27.7±1.2	34.1±4.1	0.002*
Cholesterol (mg/dL)	169.43±12.25	175.43±11.43	183.65±10.76	0.087
HDL (mg/dL)	75.80±13.62	71.65±12.34	69.59±11.76	0.230
LDL (mg/dL)	99.5±8.121	97,87±9.76	98.78±7.87	0.143
Insulin (pmol/L)	54.22±14.55	74.32±29.61	109.44±39.91	0.004*
HOMA-IR	1.58±0.51	3.23±2.43	7.89±3.77	0.001*
25-H-Vit-D (ng/mL)	29.5±8.7	18.9±7.7	12.7±6.1	0.000*

* p<0.01

Table 3. Comparison of *Apal*, *TaqI* and *BsmI* genotypes and allelic frequencies between lean, overweight and obese groups

Genotypes		Lean; n (%)	Overweight; n (%)	Obese; n (%)	p-value
Genotypes <i>Apal</i>	AA	34 (34)	41 (41)	39 (39)	0.213
	Aa	44 (44)	40 (40)	42 (42)	0.315
	aa	22 (22)	19 (19)	19 (19)	0.382
	Allele A	112 (56)	122 (61)	120 (60)	0.311
	Allele a	88 (44)	78 (39)	80 (40)	0.321
Genotype <i>TaqI</i>	TT	42 (42)	46 (46)	28 (28)	0.091
	Tt	40 (40)	41 (41)	34 (34)	0.182
	tt	18 (18)	13 (13)	38 (38)	0.021*
	Allele T	124 (62)	133 (66.5)	90 (45)	0.118
	Allele t	76 (38)	67 (33.5)	110 (55)	0.041*
Genotypes <i>BsmI</i>	BB	55 (55)	61 (61)	41 (41)	0.081
	Bb	41 (41)	32 (32)	43 (43)	0.211
	bb	4 (4)	7 (7)	16 (16)	0.042*
	Allele B	151 (75.5)	154 (77)	125 (62.5)	0.145
	Allele b	49 (24.5)	46 (23)	75 (37.5)	0.044*

* $p < 0.01$

Table 4. Comparison between BMI, HOMA-IR and 25-(OH) vitamin D in each genotypes of *Apal*, *TaqI* and *BsmI* in obese group

<i>Apal</i> genotypes	AA	Aa	aa	p-value
BMI	33.27±3.44	32.56±3.01	34.11±3.99	0.421
HOMA-IR	7.99±3.06	7.21±3.22	8.11±3.06	0.213
25-(OH) Vit D	13.14±6.32	12.66±5.02	13.55±4.77	0.138
<i>TaqI</i> genotype	TT	Tt	tt	p-value
BMI	32.11±1.14	32.24±1.38	36.18±2.11	0.048*
HOMA-IR	6.57±2.11	7.89±2.57	10.55±1.04	0.029*
25-(OH) Vit D	14.55±4.02	12.63±4.28	10.59±4.21	0.091
<i>BsmI</i> genotype	BB	Bb	bb	p-value
BMI	31.44±1.23	32.95±1.36	37.02±1.01	0.023*
HOMA-IR	6.11±1.89	7.45±2.06	10.03±1.40	0.031*
25-(OH) Vit D	15.55±3.22	15.77±2.71	16.21±2.01	0.422

* $P < 0.05$

4. Discussion

Obesity has become an epidemic problem in different areas, and its rate is increasing around the world (16). Obesity results from a combination of different factors, such as hypovitaminosis. Vitamin D deficiency is still the silent problem around the world. Several studies in different ethics have shown a high rate of vitamin D deficiency including areas with prolonged sunshine. The study by Alharbi showed that 98% of Saudi males living in Jeddah, Saudi Arabia are suffering from vitamin D deficiency (17). Different types of studies in different countries showed association between obesity and low vitamin D level. Yingshui et al., in their 2015 study on 3,867 obese Chinese subjects found an association between vitamin D deficiency and obesity (18). Another study done in 2012 by Lee and his colleagues on Korean children showed an association between low vitamin D level and both visceral obesity and hypertriglyceridemia (19). Moreover, Afzal's study (20) showed inverse correlation between vitamin D supplementation and BMI. In accordance with several studies, our results showed a lower level of 25-(OH) vitamin D in obese Saudi men compared with lean subjects. In addition, our result showed an inverse correlation of 25-(OH) vitamin D levels with BMI values and insulin levels. The role of low vitamin D level and obesity development is unclear, but some studies explain the relation between these two parameters. Kull and his colleagues (21) suggest that obese people are less exposed to sun light than non-obese people, due to reduced physical activities. Another in vitro study done by Blum et al. (22) concluded that, low vitamin D levels in obese subjects is a result of sequestration of this vitamin by adipose tissue. The VDR have been demonstrated in adipose tissue so this tissue is a response to vitamin D signals (23). Previous studies conclude that, increase 25-(OH) vitamin D level is associated with weight loss. They found that an increase in vitamin D level by 2.7 ng/mL leads to a loss of about 5%-10% of

baseline weight (24). The second factor with hypovitaminosis involved in obesity development, is genetic variations. Previously, several studies were done to determine the association of different gene polymorphisms and obesity, one of these genes involved in these studies is VDR. Vitamin D exhibits immune-modulatory and anti-proliferative effects through its receptor in disease state (25). The VDR gene is located at 12q13 chromosome and many polymorphisms are indicated at this region. The most important polymorphisms are ApaI, TaqI and BsmI which are covered by different studies in different areas and ethics (26). A previous study done by Wei-Zhen and his colleagues on a French population, found an association between BsmI genotypes and obesity (25). They found that a bb genotype is more susceptible to obesity than BB and Bb genotypes. Moreover, Al-Daghri and his colleagues (27) found an association between BsmI genotypes and obesity in a Saudi population. Fortunately, our study confirmed this association, and showed that the bb genotype is more susceptible to obesity than BB and Bb genotypes. Moreover, the bb genotype has higher BMI and HOMA-IR compared with other BsmI genotypes. On the other hand, a study done by Amar et al. (28) on Indian women did not confirm this association. According to TaqI genotypes, in 2011, a study done by Binh and his colleagues (29) on a Vietnamese population confirmed the association between TaqI genotypes and development of obesity. They found that tt genotypes have higher BMI compared with other genotypes, also their results showed a higher triglyceride value with low HDL value in tt genotype. Also, a recent study done by Saad and El-Askary (30) confirmed this association on an Egyptian population. Our results are similar to the results of these studies, we found that tt genotype is associated with obesity, and has higher BMI and HOMA-IR compared with TT and Tt genotypes. In contrast, a study done by Jakubowska et al. (31) on Polish people did not confirm this association. Finally, we did not confirm an association between ApaI genotypes and obesity, which has conflicting results to previous studies (32). Regarding the study limitations we should say that this study was conducted on Saudi male students, which represents a small size sample, so a large sample study is recommended to confirm the association of VDR polymorphism and obesity.

5. Conclusions

Our results showed a deficiency of vitamin D in young Saudi men, so vitamin D supplementation is recommended to prevent complication of this deficiency. We can conclude that there is an association between low vitamin D level and obesity in Saudi men. According to VDR genotypes, our results confirmed the association between BsmI and TaqI genotypes with obesity.

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Conflict of Interest:

There is no conflict of interest to be declared.

Authors' contributions:

All authors contributed to this project and article equally. All authors read and approved the final manuscript.

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