 Associations of Vitamin D Receptor Polymorphism rs1544410 with Adiposity Phenotypes

Abstract

**Background:** Vitamin D receptor (VDR) is present on adipocytes, and many studies were performed to investigate the association between polymorphisms in VDR gene with obesity. However, in the Arab Gulf populations, whereas obesity prevalence is increasing dramatically, only a few studies were addressed this relation with obesity based only on body mass index. This study aimed to find the association between three different VDR polymorphisms BsmI (rs1544410), ApaI (rs7975232) and TaqI (rs731236) BsmI, with the adiposity phenotypes (BMI, body fat BF% and waist circumference (WC) as a marker of visceral obesity.

**Method:** In this study, 142 young female subjects from Qatar University were recruited. The study subjects were classified into 88 control subjects (BMI < 24.9 kg/m²) with a mean age of 21.65 years and 54 overweight/obese subjects (BMI ≥ 25 kg/m²) with a mean age of 22.79 years. Blood samples and anthropometric measurements were evaluated. TaqMan assay was used to examine the genotyping of the three SNPs BsmI, ApaI, and TaqI using RT-PCR. In addition, vitamin D and insulin levels were measured using ELISA kits. The adiposity phenotypes were evaluated by anthropometric measurements of body weight, height, waist circumference and BF% were assessed by Body composition analyzer.

**Results:** The results showed that 80.3% of the study subjects were vitamin D insufficient/deficient. The main finding of the current study revealed that the carrier for the minor allele (A) in the BsmI of VDR have significantly higher BMI, WC and BF% values with p-values of 0.009, 0.015 and 0.04, respectively. In addition, it was found that increased WC is associated with lower (suboptimal) vitamin D level with an odds ratio of 3.12 and 95% CI of (1.01-9.63) with a p-value of 0.048.

**Conclusion:** The adiposity phenotype indicators including BMI, WC, and BF% were significantly associated with the minor allele (A) for BsmI (rs1544410); suggesting the possible relation of VDR polymorphism with obesity in Qatar. Vitamin D deficiency could affect the BF% in overweight and obese subjects.

**Keywords:** Overweight; Obesity; Adiposity phenotype; VDR; Vitamin D; Qatar; Cardiovascular diseases; Hypertension; Type 2 diabetes

**Abbreviations:** BMI: Body Mass Index; BF%: Body Fat Percentage; WC: Waist Circumference; NOW: Non-Overweight; NOB: Non-Obese; OB: Obese; OW: Overweight; OR: Odds Ratio; SNP: Single Nucleotide Polymorphism; VDR: Vitamin D Receptor

**Introduction**

The prevalence of obesity is rising worldwide, and presenting a public health problem. According to the World Health Organization (WHO), the obesity epidemic has more than doubled between 1980 and 2014. In 2014, over 1.9 billion adults (18+ years) were overweight, with over 600 million being obese (http://www.who.int/mediacentre/factsheets/fs311/en/). Obesity is associated with major health problems such as cardiovascular diseases, hypertension, type 2 diabetes, gall bladder disorders, and cancers [1].

Obesity is a multifactorial chronic disease of both genetic and environmental interpositions, whereas many genes have been known to play a role in the pathogenesis of the diseases (Clément & Ferré, 2003). Several studies demonstrated the role of vitamin D in obesity through different genetic, experimental, epidemiological and metabolic data linking adipokines with Vitamin D [2-5]. Vitamin D produces its effects through its binding to vitamin D receptor (VDR); a nuclear transcription factor [6]. The vitamin D receptor (VDR), is produced by the VDR gene locus (VDR) on chr12q13.1, a member of the steroid hormone receptor superfamily [7]. Previous studies proposed that both 1,25(OH)2D3 and VDR both contribute a significant role in adipocyte differentiation [5,8,9]. Previous studies disclosed that 1,25(OH)2D3, through its anticipated interactions with VDR, repressed the early differentiation of preadipocytes to mature adipocytes in 3T3-L1 cells [8,10]. Experimental studies showed adipose atrophy surrounding the mammary and prostate tissue in VDR knockout mice [9,10]. Other investigators demonstrated that the action for VDR in adipogenesis is based on the effect of VDR messenger RNA concentrations during adipocyte differentiation.
Qatar 2016 survey showed that 76.6%, 38.9% and 76.6%, 47.8.2% of adult men (18-64Y) and women (18-64Y) are overweight and obese respectively (http://www.who.int/diabetes/country-profiles/qat_en.pdf?ua=1 accessed on 28 October 2016). This dramatic surge in the obese population conveys substantial health apprehensions and imposes a financial load on countries throughout the world. Moreover, recent studies showed 61.6% of Qatari adolescence are at high risk for vitamin D deficiency [16] and a high prevalence of vitamin D deficiency and insufficiency (97.2%) among healthy college female subjects [17].

Therefore, we investigated the genetic association relation between three variants of VDRs; rs1544410 (BsmI), rs7975232 (ApaI) and rs731236 (TaqI) with the adiposity phenotypes (BMI, WC and %BF) in overweight and obesity in young Arabian female subjects in Qatar, a country where overweight and obesity and Vitamin D reached endemic proportions.

Subjects and Methods

Study subjects

This is a prospective cross-sectional study. In this case-control association study, 142 young females from Qatar University were enrolled in this study. The adiposity phenotype of the participants was evaluated by anthropometric measurements, and blood samples were drawn for genotyping and biochemical assays.

Qatar University female students were recruited to encourage the students to participate in this study, such as participating in a health event about Vitamin D and obesity. The event was advertised via QU-email and social media network that includes Facebook and WhatsApp. Students who decided to participate in the study were informed about the aim, requirements, benefits and risks of the procedure that are included in the study. After that student was asked to read and sign a consent form and answer a questionnaire. Approval to conduct this study was granted by the Ethical Committee of Qatar University board in accordance with the Declaration of Helsinki [18]. The exclusion criteria included the following if the subject received vitamin D supplement, calcium supplements in the last three months, or has a pregnancy, lactation, liver, renal immune disorders and cancer.

Adiposity phenotype measurements

Personal data were recorded such as age, medical history, and family history to ensure the participant do not violate follows the exclusion criteria. Body weight was measured using a centrally purchased electronic scale [Seca] with accuracy to 0.1kg following a standardized technique (lightly dressed, without shoes). Standing height was measured with the use of a stadiometer, with the shoulders in relaxed position and arms’ hanging freely to the nearest 0.1cm. Body mass index “BMI” was calculated by dividing weight in [Kg] by height squared meter [m^2]. Waist (WC) was measured in duplicate with the subjects standing dressed in light clothes. WC was measured by means of Seca ergonomic circumference measuring tape with subjects standing at the smallest abdominal position between the iliac crest and the lower rib margin at the end of normal expiration. The measurements were recorded to the nearest 0.5cm. Correct posture of the subject was ensured by maintaining the Frank Front plane. Body composition analysis was performed by each student using (InBody 720) body composition analyzer. These measurements include body fat mass and percent body fat. All anthropometric measurements were evaluated in the Nutrition Department-Qatar University by trained instructors. BMI categorized into the following categories: underweight (<18.5), normal weight (18.5-24.9), overweight (25-30), and obese (>30) [19], % body fat for females >30% is used as a cut-off point to define obesity [20] and if waist circumference (WC) >88cm is used to define central obesity [21]. The homeostatic model assessment (HOMA) is used to quantify insulin resistance and beta-cell function, whereas HOMA-IR=[Glucose in mmol/l] * [Insulin in mU/L]/22.5 [22].

Biochemical measurements

After overnight fasting, 10ml venous blood samples were withdrawn for genotyping and biochemical assays for metabolism, vitamin D, and insulin. EDTA-containing tubes were used to collect blood for Genotyping. Measurements of biochemical analysis included the lipid profile, glucose while vitamin D level and insulin were measured by Elisa technique in biomed lab Sciences-Qatar University as previously published [23]. The concentration of fasting insulin was performed using the insulin ELISA kit Quantikine ELISA kit (catalog DINS00, USA), and Vitamin D was measured by 25-OH vitamin D (total) ELISA Cat#5396 (DRG Diagnostics, USA). The assay was based on the manufacturer’s instructions by the supplied company for each assay.

Selection of VDR SNPs and genotyping

The genetic analysis was performed in a biomedical research center at Qatar University. In brief, DNA was extracted using a venous blood sample collected in EDTA tubes from all participating patients, the DNA extraction kit used was (Qiagen, EZ1 DNA Blood 350µl Kit, Cat. No. 951054). Three SNPs were selected for this study was (rs731236, rs7975232, and rs1544410) as previously published in the literature [13,24]. The polymorphisms of the SNPs were analyzed by the 5’ nuclease assay using TaqMan MGB probe using an ABI 7500 (Applied Biosystems, Foster City, CA). The 5’ nuclease assay was performed by adding 23µl of the solutions prepared (TaqMan master mix, 40x SNP and H2O) to 2µl of the patient’s DNA sample, this yield a total volume of 25µl in each well. The assay-on-demand TM service provided the primers and the probes of these SNPs polymorphisms by (Applied Biosystems). The proper conditions for amplifications were followed by the manufacturer’s instructions and negative controls were used, as previously published [23].

Statistical analysis

Data were explored for outliers, skewness, and normality and transformed when necessary if normality assumption was violated. Continuous data are expressed as mean ± SD for normally distributed variables, median and inter-quartile range [25%-75%] for non-distributed continuous data and number and (percentage) for categorical data. Two student's t-tests and non-parametric Mann-Whitney evaluated the differences between

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[11,12]. Several studies in human subjects showed an association of VDR variants with obesity phenotypes [13-15].
continuous variables, and 2-independent samples t-tests were used accordingly for analysis. Differences between categorical variables were assessed by chi-square test. Genotype distributions and allele frequencies between the study groups were compared by constructing 2X2 contingency tables, \(x^2\), and/or Fisher exact test corrected for Bonferroni adjustment for the number of SNPs. The Hardy-Weinberg equilibrium (HWE) was calculated using the chi-square test to determine genotype distribution in all study subjects. Odds ratios (ORs), 95% confidence intervals (CIs), and corresponding \(P\) values for the samples were analyzed by logistic regression analysis. ORs were computed using the homozygous minor alleles as the reference group unless otherwise stated.

All statistical analyses were performed using the SPSS program for Windows (version 21 statistical software; Texas Instruments, IL, USA), graph pad prism (version 6, for Mac, Graph Pad Software, La Jolla California USA) and the Golden Helix SNP and Variation Suite (SVS 8, software; Bozeman, MT, USA) for genetic analysis. Two-tailed \(P\) value \(<0.05\) is statistically significant.

### Results

#### Clinical features of study subjects

The study subjects were divided into two categories according to their body mass index measurements. The first group was lean subjects (\(n=88\) subject, \(\%61.97\)) with BMI \(<25\text{kg/m}^2\), while the second group included both overweight and obese individuals (\(n=54\) subjects, \(38.03\%\)) with BMI \(\geq 25\). Data in Table 1, showed that overweight (OW) and obese (OB) individuals have significantly higher mean and standard deviation (SD) values for; BMI (\(P =<0.001\)), waist circumference (P\(=<0.001\)), percent body fat (\(P=<0.001\)) triglycerides (\(P=0.031\)), insulin (\(P=<0.001\)) and HOMA-insulin resistance (HOMA-IR) (\(P=0.001\)). On the other hand, OW+OB subjects had significantly lower levels of high density lipoprotein cholesterol (HDL-C) than lean subjects with a \(p\) value \(<0.001\). However, the insignificant difference was observed for the following variables: age (\(p=0.058\)), glucose (\(P=0.345\)), total cholesterol (\(p=0.067\)), low density lipoprotein cholesterol (LDL-C) (\(p=0.467\)) and vitamin D level (\(p=0.747\)).

#### B-VDR genetic distribution and its association with overweight and obesity based on BMI

Quality control assessment for the three studied SNPs is shown in Table 2. All SNPs have shown call rate values >85%. Also, all studied SNPs were evaluated in all the study subjects using the Hardy-Weinberg equilibrium, only both rs1544410, and rs7975232 are within the equilibrium \((p\geq0.05)\). In contrast, rs731236 showed a deviation from the Hardy-Weinberg equilibrium \((p=7.761e-015)\), thus it was not used for further analysis in this study.

Genotypic distribution of all SNPs is presented in Table 2. It showed that the heterozygous AG genotype is the predominant in distribution in OW+OB and lean groups. The genotype AG of rs1544410 in all subjects is \((n=63, 44.7\%)\), in overweight/obese subjects \((n=27, 50.0\%)\), and in lean subjects \((n=36, 41.4\%)\). For the SNP rs797232, the AC genotype is the predominant in distribution among the studied subjects. In all subjects, the AC genotype \((n=61, 44.8\%)\). Overweight/obese subjects have \((n=24, 47.2\%)\), while lean subjects have \((n=37, 43.5\%)\) genotypic count and percentage. No significant differences in genotype

#### Table 1: The study characteristics in overweight (OW) and obese (OB) and non-overweight (NOW) and Non obese subjects (NOB).

| Variables                  | OW+OB Subjects (n=54) | NOW+NOB Subjects (n=88) | \(P\)-value |
|----------------------------|-----------------------|-------------------------|-------------|
| Age (years)                | 22.79 (4.48)          | 21.65 (2.64)            | 0.06        |
| BMI (kg/m\(^2\))          | 29.781 (4.05)         | 21.055 (2.410)          | \(<0.001\)  |
| Waist Circumference (cm)  | 93.17 (9.51)          | 77.060 (7.6628)         | \(<0.001\)  |
| Percent Body Fat (%)       | 43.39 (5.34)          | 31.30 (6.12)            | \(<0.001\)  |
| Glucose (mM)              | 4.95 (0.49)           | 5.02 (0.40)             | 0.345       |
| TC (mM)                   | 3.93(0.731)           | 4.21 (0.92)             | 0.067       |
| LDL-C (mM)                | 2.30 (0.58)           | 2.39 (0.73)             | 0.476       |
| HDL-C (mM)                | 1.23 (0.31)           | 1.47 (0.35)             | \(<0.001\)  |
| Triglycerides (mM)        | 0.86 (0.35)           | 0.75 (0.25)             | 0.031       |
| Insulin (ml/ml)           | 21.14 (14.61-38.99)   | 18.41 (3.47-33.63)      | \(<0.001\)  |
| HOMA-IR (AU)              | 9.68 (4.65-22.86)     | 3.69 (0.72-8.14)        | \(<0.001\)  |
| Vitamin D (ng/ml)         | 14.97 (10.53-19.42)   | 16.25 (12.73-19.77)     | 0.654       |

Data are presented as mean and (SD) for continuous normally distributed data and mean and (95% lower and upper bound of CI of the mean) for non-normally distributed data. Independent sample t-test and Mann-Whitney tests are used for analysis accordingly. Two-tailed \(P\) value is significant at \(\leq0.05\).

TC: Total Cholesterol; HDL-C: High-Density Lipoprotein Cholesterol; LDL-C: Low-Density Lipoprotein Cholesterol.
distributions between both groups for both rs1544410 and rs7975232 with (p=0.400) and (p=0.830), respectively. The minor allele was A for rs1544410, with the frequency of 0.491, and 0.425 in OW+OB and lean subjects respectively. The minor allele was C for rs7975232, with the frequency of 0.314, and 0.371 in OW+OB and lean subjects respectively.

**Table 2:** Genotype distribution and minor allele frequency in OW+OB group versus NOW+NOB group.

| SNP's    | Genotype | All Subjects (n=141) | OW+OB (n=54) | NOW+NOB (n=87) | P-value |
|----------|----------|----------------------|--------------|----------------|---------|
| rs1544410| AA       | 32 (22.6%)           | 13 (24.1%)   | 19 (21.8%)     | 0.400   |
|          | AG       | 63 (44.7%)           | 27 (50.0%)   | 36 (41.4%)     |         |
|          | GG       | 46 (32.6%)           | 14 (25.9%)   | 32 (36.8%)     |         |
|         | Minor Allele Frequency (A) | 0.450 | 0.491 | 0.425 |
| rs7975232| AA       | 56 (41.2%)           | 21 (41.2%)   | 35 (41.2%)     | 0.830   |
|          | AC       | 61 (44.8%)           | 24 (47.2%)   | 37 (43.5%)     |         |
|          | CC       | 19 (14.0%)           | 6 (14.6%)    | 13 (15.2%)     |         |
|         | Minor Allele Frequency (C) | 0.364 | 0.314 | 0.371 |

Data are presented as number (%) for genotype counting in all subjects, OW+OB and NOW+NOB subjects, while MAF is portrayed as a fraction. Data were analyzed by chi-square test. Two-tailed p value is significant at ≤0.05.

**Vitamin D level with the genotype distribution of rs1544410 & rs7975232**

Moreover, the vitamin D level was evaluated with the individual genotyping and the dominant genotyping models of each SNP (rs1544410 & rs7975232). The data displayed in Table 3, the mean values and 95% CI of the mean. No significant difference was observed for vitamin level among the individual genotyping and the dominant genotype models (p >0.05).

**Table 3:** Vitamin D level based on the genotype distribution of rs1544410 & rs7975232.

| Genotype | Vit D (ng/ml) | P-value |
|----------|---------------|---------|
| rs1544410|               |         |
| AA*      | 13.67 (9.23-18.11) | 0.597   |
| AG       | 16.55 (12.46-20.46) |         |
| GG       | 16.92 (10.03-23.81) |         |
| AG+AA*   | 15.33 (10.35-18.31) | 0.634   |
| GG       | 16.92 (10.03-23.81) |         |
| rs7975232|               |         |
| AA       | 15.95 (11.26-20.68) |         |
| AC       | 15.62 (11.59-19.65) |         |
| CC*      | 17.01 (8.44-25.95)  | 0.951   |
| CC+AC*   | 15.94 (12.36-19.54) | 0.998   |
| AA       | 15.95 (11.26-20.68) |         |

**Stratification of vitamin D level categories with the adiposity phenotype indicators**

In addition, the adiposity phenotype indicators that included the waist circumference, the percent body fat (BF %) and BMI were used to assess the association with vitamin D categories (optimal ≥30ng/ml versus suboptimal <30ng/ml. The analysis aimed to determine if obesity is associated with subjects having low vitamin D level or not. Table 4, presents the Odds ratio and 95% of CI with their p values of obesity using the different phenotype markers (BMI, WC and BF%) among the study subjects that were categorized based upon the cut-off value for vitamin D level as optimal (≥30ng/ml) and suboptimal (<30ng/ml). Only the subjects with low vitamin D level (insufficiency and deficiency)
had high waist circumference values (≥88cm) and an odds ratio of 3.12 with 95% CI of (1.01-9.63) and a p-value of 0.048. Other associations for adiposity phenotype indicators (BMI and BF %) among low vitamin D level subjects showed insignificant associations with p values (0.475, and 0.700), respectively.

Genotypic distributions of both SNPs rs1544410 & rs7975232 and obesity indicators among obese individuals

Further analysis was performed to investigate the associations of the genotype distributions with these adiposity phenotype markers. Genotypic distribution of both SNPs was performed in parallel to the adiposity phenotype indicators (BMI, WC, and BF %) as shown in (Tables 5, 6 & 7). Individuals carrying the genotype AA or AG for the SNP (rs1544410) have a significantly higher mean value of BMI (kg/m^2) (25.5) in comparison to individuals who are homozygous for the major allele (GG) who had a mean value of (22.7) with a p-value of 0.009. The genotypic distribution for the SNP (rs7975232) had no significant difference between mean values of BMI for both models. When both SNPs were analyzed in parallel to percent body fat (BF%), all models had insignificant difference between mean values of the percent body fat, except for (rs1544410) dominant model that had significantly higher BF% mean value (36.9) with a p-value of 0.04. Furthermore, a significant difference was observed between WC mean values for individuals who carry AA or AG genotype for the SNP (rs1544410) with a mean value of (84.9) versus individuals who are homozygous for the major allele (GG) who had a mean value of (79.87) with a p-value of 0.015. In contrast, no significant difference was observed in WC for all models of the SNP (rs7975232) with BMI.

Effect of pairwise interaction of VDR variant with Vitamin D level on adiposity phenotype markers

Using the regression analysis between the VDR variant in a dominant model with the vitamin D level to study its effects on the distinct adiposity phenotypes; BMI, WC, and BF%. As shown in the table 8, only the pairwise interaction of vitamin D level with a variant in the dominant model for rs1544410 in its genetic model has significant effects on central obesity (WC) with p values <0.05, while other pairwise interactions on BMI and BF% are not significant p >0.5.

Table 4: Association between Vitamin D level categories with obesity by the adiposity phenotypes; BMI, WC and BF% categories.

| Variable | Odds Ratio | OR Lower Conf. Bound | OR Upper Conf. Bound | P value |
|----------|------------|----------------------|----------------------|---------|
| OW/OB   | 1.37       | 0.57                 | 3.13                 | 0.475   |
| WC      | 3.12       | 1.01                 | 9.63                 | 0.048   |
| BF%     | 1.24       | 0.42                 | 3.71                 | 0.700   |

Data are presented as odds ratio with its 95% lower and upper confidence interval (CI) along with their p values among vitamin D insufficient and deficient subjects versus normal subjects. Data were analyzed by logistic regression analysis. Two-tailed p value is significant at ≤0.05.

Table 5: Genotype distribution of rs1544410 & rs7975232 and its relation with BMI level among study subjects.

| Genotype | BMI | P-value |
|----------|-----|---------|
| rs1544410 |     |         |
| AA       | 25.409 (6.00) | 0.031   |
| AG       | 25.102 (4.95) |         |
| GG       | 22.73 (4.89)  |         |
| AG+AA    | 25.5 (5.32)   | 0.009   |
| GG       | 22.7 (4.89)   |         |
| rs7975232 |     |         |
| AA       | 25.057 (6.02) | 0.184   |
| AC       | 24.269 (4.75) |         |
| CC       | 22.458 (4.65) |         |
| CC+AC    | 23.8 (40.80)  | 0.191   |
| AA       | 25.06 (6.02)  |         |

Data for BMI (kg/m2) are shown as a mean value with (standard deviation). Genotypic distribution was found for both additive and dominant models using one way-ANOVA followed by a post-hoc test. Two-tailed p value is significant at ≤0.05.

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Table 6: Genotype distribution of Rs1544410 & Rs7975232 and its relation with percent body fat (BF%).

| Genotype | Percent Body Fat | P-value |
|----------|-----------------|---------|
| rs1544410 |                 |         |
| AA       | 36.834 (8.97)   | 0.121   |
| AG       | 36.998 (7.31)   |         |
| GG       | 33.885 (8.84)   |         |
| AG+AA    | 36.90 (7.87)    | 0.04    |
| GG       | 33.89 (8.85)    |         |
| rs7975232 |                 |         |
| AA       | 36.51 (9.07)    | 0.698   |
| AC       | 35.80 (7.45)    |         |
| CC       | 34.64 (9.29)    |         |
| CC+AC    | 35.50 (7.90)    | 0.503   |
| AA       | 36.50 (9.10)    |         |

Data for %BF are shown as a mean value with (standard deviation). Genotypic distribution was found for both additive and dominant models using one way-ANOVA followed by a post-hoc test. Two-tailed p value is significant at ≤0.05.

Table 7: Genotype distribution of Rs1544410 & Rs7975232 and its relation with waist circumference.

| Genotype | Waist Circumference | P-value |
|----------|---------------------|---------|
| rs1544410 |                     |         |
| AA       | 85.24 (13.19)       | 0.05    |
| AG       | 84.68 (11.49)       |         |
| GG       | 79.87 (9.63)        |         |
| AG+AA    | 84.90 (12.02)       | 0.015   |
| GG       | 79.87 (9.63)        |         |
| rs7975232 |                     |         |
| AA       | 84.15 (12.52)       | 0.361   |
| AC       | 83.25 (11.52)       |         |
| CC       | 79.73 (8.95)        |         |
| CC+AC    | 82.40 (11.02)       | 0.396   |
| AA       | 84.15 (12.5)        |         |

Data for WC are shown as a mean value with (standard deviation). Genotypic distribution was found for both additive and dominant models using one way-ANOVA followed by a post-hoc test. Two-tailed p value is significant at ≤0.05.

Table 8: Pair wise interaction of rs1544410 & rs7975232 with Vitamin D on different adiposity phenotypes in the study subjects.

| Interaction with Vitamin D | Coefficient | Std. Error | Alternatively, and 95%% CI | P     |
|---------------------------|-------------|------------|-----------------------------|-------|
| BMI (OW/OB)               |             |            |                             |       |
| rs1544410 (AG+AA vs. GG)  | 0.006       | 0.011      | 1.01 (0.99-1.03)            | 0.598 |
| rs7975232 (CC +AC vs.CC)  | 0.021       | 0.013      | 1.03 (1.01-1.05)            | 0.058 |
| WC                        |             |            |                             |       |
| rs1544410 (AG+AA vs. GG)  | 0.029       | 0.014      | 1.04 (1.01-1.07)            | 0.029 |
| rs7975232 (CC +AC vs.CC)  | 0.003       | 0.001      | 1.00 (0.94-1.02)            | 0.817 |
| Total BF %                |             |            |                             |       |
| rs1544410 (AG+AA vs. GG)  | 0.007       | 0.014      | 1.01 (0.95-1.02)            | 0.617 |
| rs7975232 (CC +AC vs.CC)  | 0.003       | 0.001      | 1.00 (0.94-1.02)            | 0.8   |

Data are presented as intercept (coefficient), the standard error for the constant, Odds ratio with its 95% lower and upper confidence interval (CI) along with their p values. Data was analyzed by logistic regression analysis. Two-tailed p value is significant at ≤0.05.
Discussion

Overweight and obesity are major health problems that are widely distributed around the world. According to recent statistics, Qatar has a high prevalence of overweight and obesity among adults that reached to 76.6% and 41.0% respectively (WHO, 2016). Obesity is considered as independent risk factor for many serious diseases such as metabolic, cardiovascular, respiratory and gastrointestinal disorders [25]. Due to the serious complications of obesity and its high prevalence in Qatar in parallel with the endemic proportion of vitamin D deficiency and insufficiency; the present study was conducted on Arabian young female subjects as a target whereas the lifestyle factors could be limited, and the genetic factors could be more evident in this young age group.

This observational study aimed to find a genetic association between VDR variants and the risk for obesity assessed by three markers for adiposity phenotypes (BMI, WC, and BF%). Few studies were performed concerning such genetic association in the Arabian Gulf Area with obesity using only BMI as adiposity phenotype [13].

The main findings of the study showed that carriers of the minor allele A in rs1544410 is associated with the increased risk of getting obesity as indicated by the three adiposity indicators (BMI, BF%, and WC). The finding of the present study is in agreement in general with Previous human studies demonstrated that VDR polymorphism on chromosome 12, q13 could be linked to obesity [13-15]. Moreover, decreasing vitamin D level below the optimal (30ng/ml) is associated with the increased %BF and adiposity. These findings will be discussed further in the following paragraphs.

The results of the current study showed that carriers of the minor allele (A) for the SNP BsmI (rs1544410) are at higher risk of having increased BMI in comparison to individuals who are homozygous for the major allele (G). A previous study was performed in Saudi Arabia had similar findings, which demonstrated that the minor allele for the SNP BsmI is more frequent in obese individuals [13]. However, polymorphism in SNP TaqI (rs731236) showed increased BMI in minor allele carriers (C), which in not consistent with data for rs731236 of the current study. On the other hand, another study in Greek population showed a significant increase in BMI in individuals who carry the major allele (T) for the SNP TaqI (rs731236) [15], which is different from the data of the current study. These findings conflict the results of this study as TaqI was deviating from HWE and was not further analyzed. This could be due to the limited sample size, the homogeneity of the population (only female subjects) and the different genetic background (Arab vs. Greek) used in this current study.

Furthermore, VDR polymorphism in SNP BsmI (rs5144410) is associated with increased WC, a reflection of central obesity in human subjects. The present study showed that individuals who carry the minor allele (A) for BsmI had a significant risk for having increased central or visceral obesity. The association between VDR polymorphism in BsmI and higher WC was observed in a previous study that was implemented in North China, but the difference was that individuals who are at risk were carrying the major allele (G) [24]. This difference could be due to the limited number of samples in the current study and the homogeneity of the population in the present study and the ethnic background of the population under each study.

Also, the percent body fat was also related to VDR polymorphism in BsmI minor allele (A) in the current study. In conflict with the current findings for %BF with BsmI, a study in Vietnam using the same SNP did not detect a significant relation between %BF and VDR polymorphism [26]. This contradiction could be due to the difference in the population age, stratification and the ethnic background. Furthermore, the current data showed no significant association for rs7975232 with obesity, which is consistent with the previous study among Saudi subjects, of the same ethnic background [13].

The explanation of the association between the BsmI rs1544410 with the increased risk of getting obesity as indicated by the three adiposity indicators (BMI, BF%, and WC), is not yet fully understood. VDR variant BsmI may impact the binding of 1,25(OH)2D to VDR and further could mediate a mass of downstream effects on VDR-responsive genes. The effect of VDR BsmI variant polymorphism could be direct mediating effects through the immune system and inflammatory pathway by alteration of the inflammation is possibly resulting from the gut microbial translocation [13,27]. In addition, The BsmI, and TaqI polymorphism are located in the 3’ untranslated region (3’ UTR) of the VDR gene. The BsmI SNP are located in intron 8, and TaqI is a silent SNP in exon 9, which are involved gene expression modulation [28]. Other explanation showed that the action for VDR in adipogenesis is based on the effect of VDR messenger RNA during adipogenesis [11,12]. The functional role of the BsmI and TaqI VDR gene polymorphisms need further investigations.

Further, the current data indicated that subjects with low vitamin D level below the optimal (30ng/ml) at high risk of developing central obesity (WC). This finding is in agreement with previous studies showing subjects with low vitamin D level had increased visceral fat area in Chinese men [29] and lower levels of 25(OH)D were associated with higher adiposity measures [30,31]. This finding of central obesity observed in the current study could be attributed to sequestration of vitamin from skin and dietary sources into body fat partitions, with a consequent decrease in the bioavailability of vitamin D3 [31,32]. Several mechanisms were postulated to explain the reduced levels of serum vitamin D with adiposity phenotype by increased visceral fat. Reduced Vitamin D level can cause elevation of parathyroid hormone (PTH), which increase lipogenesis and reduce lipolysis and through suppression of PPAR expression in adipocytes [9,33,34].

Our data showed no effect of the genetic variants on vitamin D level in the study subjects. Few studies studied the relation between VDR polymorphisms and 25(OH)D levels, particularly in young and healthy subjects. In a study with adolescent girls, “the Bsm1 polymorphism was marginally, but not significantly, related with 25(OH)D levels” [35]. In another study, TaqI polymorphism was linked with higher 25(OH)D levels in healthy adults of old age [36], although in another study BsmI and TaqI SNPs demonstrated no significant association with vitamin D concentrations [37].
Furthermore, we did pairwise interaction to detect the interface effect of vitamin D and VDR genotyping on adiposity phenotype markers particularly WC. The data of the current study demonstrated a significant positive interaction of rs1544410 with circulating 25-hydroxyvitamin D concentrations. This data could refer to the synergistic effect of both factors on mediating a host of effects on VDR-responsive genes. The independent individual effect of vitamin D alone and the VDR Bsm1 variant on central obesity (WC) could refer to the complicated effects of 25-hydroxyvitamin D, VDR and VDR-responsive genes in mediating obesity especially the visceral fat. Previous studies proposed that both 1,25(OH)2D3 and VDR both contribute a significant role in adipocyte differentiation [5,8,9]. Previous studies disclosed that 1,25(OH)2D3, through its anticipated interactions with VDR, repressed the early differentiation of preadipocytes to mature adipocytes in 3T3-L1 cells [8,10]. VDR may exert its independent effects from those of 1,25(OH)_{2}D_{3} [10]. Though, upcoming studies are required to explore this multifaceted interaction of vitamin D and VDR polymorphisms to the downstream influence on adiposity-related endpoints especially the visceral obesity.

**Conclusion**

This observational, case-control study was conducted to highlight the association between VDR polymorphism and adiposity phenotypes of obesity. Body mass index, waist circumference and percent body fat that are adiposity indicators were significantly higher in individuals carrying the minor allele (A) for Bsm1 (rs1544410); suggesting the possible relation of VDR polymorphism and vitamin D with obesity in Qatar.

**Limitations**

The major limitations of the current study are the limited sample size and the age range, the and homogeneity of the population (only female subjects).

**Recommendation**

Studies with larger sample size and more SNPs in VDR region including both genders is recommended. The role of inflammation in parallel to VDR polymorphism in obesity can be further studied which help in understanding the pathogeneses of obesity.

**Prospective**

The role of inflammation in VDR polymorphism and obesity can be further explored in a wider range. Moreover, tissue culture techniques can be used to find the cause/effect relationship between vitamin D and obesity. Studies with large samples size, with more SNPs in VDR region and both genders, is recommended.

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