Association of Vitamin D levels and Vitamin D receptor gene polymorphism with obesity in Bangladeshi school-going children: A case-control study

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Abstract

Background:

Childhood obesity and vitamin D deficiency (VDD) are recent health concerns associated with several clinical, psychosocial, and genetic manifestations like cardiovascular diseases, diabetes, depression, and cancer. This study aimed to investigate the association between lifestyle variables and vitamin D levels and VDR gene polymorphism with obesity among Bangladeshi school-going children.

Methods:

Epidemiological data and blood samples were collected from a total of 164 participants aged 6-13. Serum vit-D level was measured using electrochemiluminescence immunoassay (ECLIA) and four single nucleotide polymorphisms (SNPs) of the VDR gene such as TaqI, BsmI, Apal, and FokI were genotyped by polymerase chain reaction-restriction fragment length polymorphism assay (PCR-RFLP).

Results:

The vit-D level was significantly lower in obese children (37.54 ± 14.39 ng/mL) compared to the underweight, normal, and overweight groups (44.08 ± 15.57 to 50.46 ± 19.25 ng/mL) (p=0.013). Among the lifestyle variables, sunlight exposure during the daytime had a significant effect on the vit-D level of the participants regardless of their BMI status (p=0.003). The SNPs of the VDR gene study demonstrated that the Bb allele of the BsmI gene in obese children (58.62%) was significantly different from the control groups (73.33%) (p=0.02). 24.14% of obese children were of BB genotype, 58.62% of Bb genotype, and 17.24% of bb genotype, while in controls, BB, Bb, and bb genotypes were 20%, 73.33%, and 6.67%, respectively. Importantly, 66.67% of children with vit-D deficiency were BsmI-bb genotype carriers whereas only 5% of children were BsmI-bb genotype carriers who had sufficient vit-D concentrations.

Conclusion:

A significant association of reduced vitamin D levels and Bb alleles of the BsmI with childhood obesity has been identified. Hence, reduced vitamin D levels and VDR-BsmI polymorphism are risk factors for childhood obesity and suggest further study with a larger number of participants and lifestyle as well as therapeutic interventions in obese children.

Highlights

- Daily sunlight exposure for at least one hour maintains the standard vitamin D (vit-D) level requirements of all BMI group children.

- Childhood obesity is significantly associated with a lower level of vit-D.
Obese children carry Bb alleles of the BsmI genotype significantly and 66.67% of children with vit-D deficiency were BsmI-bb genotype carriers.

There is no significant association between the Vit-D level and VDR polymorphism among all the BMI group children.

Introduction

Obesity in children is an emerging public health concern in developing countries like Bangladesh and the prevalence of this disease is upsurging alarmingly\(^1\). According to the WHO, childhood obesity is a major public health challenge for the 21st century and it is linked to several psychosocial consequences such as lower self-esteem, social isolation, poor academic achievement, and peer problems. Obesity in children is also correlated with cardiovascular diseases, Type 2 diabetes (T2D), and dyslipidemia which are well-established illnesses\(^1\). Vitamin D is required for the development and maintenance of bone tissue, for maintaining the normal level of calcium and phosphorus in the body, alongside for cell differentiation, proliferation, and hormone secretion\(^2\).

Vitamin D is a fat-soluble secosteroidal hormone found in the diet or synthesized in the skin when 7-dehydrocholesterol reacts with sunlight. The biologically inactive vitamin D3 is hydroxylated in the liver and kidney into active calcitriol, (1,25(OH)\(_2\)D). The concentration of vitamin D below 20, 21–29, and 30–100 ng/mL indicates vitamin D deficiency, insufficiency, and sufficiency, respectively\(^3\).

Studies have shown that there is a negative correlation between serum vitamin D level (25-OH D) and body fat. Vitamin D (vit-D) is active in the fat tissues and coupling of vitamin D with vitamin D receptor (VDR) exerts multiple biological functions and plays a crucial role in the regulation of gene expression. Serum vitamin D deficiency accounts for increased obesity by reducing VDR function. Study findings have shown that over-expression of the human VDR gene in adipocytes is linked to reduced energy expenditure and induction of obesity\(^2\). Previous findings have also shown that VDR variants are associated with adipocytes phenotypes and these variations in the DNA sequence are known as polymorphisms. The VDR gene contains several polymorphisms including single nucleotide polymorphisms (SNPs); Taq1, Bsm1, Apa1, and FokI identified by their restriction endonuclease sites. Furthermore, VDR mRNA stability can be influenced by a poly (A) microsatellite which is linked to SNPs if they become functional\(^4\).

VDR gene has been reported to be significantly associated with obesity among the Iranian population\(^5\). On the other hand, a study on the association of VDR polymorphism with the diseases like chronic diabetes mellitus, hypertension, and obesity among UAE participants revealed no significant correlation except with the vit-D concentration\(^6\). However, investigations on Chinese, Caribbean, and Iranian populations showed a significant association of VDR polymorphisms with increasing susceptibility to causing periodontitis, urticarial and COVID-19 infection respectively\(^7\)–\(^9\). Another research among Brazilian school-going children showed that VDR gene polymorphisms are responsible for asthma in both normal
and overweight children. They also reported that in general, the AA alleles of FokI function as the risk factor for asthma whereas GG alleles were protective for the children. In addition to this, normal-weight children showed a higher risk of asthma having the polymorphic TT alleles of ApaI and overweight children were under risk with the BsmI gene polymorphism. VDR gene polymorphisms and their association with obesity and other clinical conditions have been investigated vastly among adults. However, relevant research on children is still in limited numbers and children should be considered as a more potential subject group so that they can be protected from future clinical conditions caused by vit-D deficiency and VDR gene polymorphisms.

Serum level of vitamin D among Bangladeshi children and infants was determined previously. However, no study was conducted previously on the correlation of vitamin D level and vitamin D receptor gene polymorphism with obesity in Bangladeshi school going children. Therefore, the aim of our study was to identify the association of serum vitamin D level and single nucleotide polymorphisms (SNPs) of VDR gene with obesity-related anthropometric measures such as weight, height, waist circumference, triceps skinfold (TS), and subscapular skinfold (SSS) for body fat percentage in Bangladeshi school going children.

Methods

Ethical permission

Ethical permission was sought from the Institutional Review Board (IRB), BRAC University (IRB Reference No. FRF01/2021-5). All methods were performed in accordance with the relevant guidelines and regulations mentioned in the approved research protocol.

Study design and data collection

This cross-sectional study was carried out with children of 6–13 years of age, regularly enrolled in public or private schools inside the Dhaka metropolitan city and adjacent areas. Participant exclusion criteria was a clinical history of chronic diseases such as bone diseases, diabetes, liver and kidney diseases, presence of acute infectious or inflammatory processes, and oral corticosteroids use at the time of data collection or others (except obesity).

Development of questionnaire

In order to assess children's exposure to vitamin D, a structured questionnaire covering aspects regarded as pertinent to vitamin D exposure was developed by reviewing other vitamin D exposure related research. Questionnaire pre-test and validity test were performed. The questionnaire of this study focused on sources of vitamin D from sunlight, foods, fishes, supplements. This questionnaire also focused on the clinical history of the participants and their families; those may be responsible for the deficiency of vitamin D absorption.
Recruitment of the participants and fill-in the questionnaire

The importance of the study was explained to the potential participants and to their guardians. The participants were recruited by signing the consent form by their legal guardian who agrees. The structured questionnaire was answered by one of their legal guardians and was filled-in by the trained research Assistants of this study.

The anthropometric measurements were performed as recommended by the World Health Organization (WHO) and Frisancho\textsuperscript{15,16}, including measures of weight, height, waist circumference, triceps skinfold (TS), and subscapular skinfold (SSS) for body fat percentage.

**Determination of Vitamin-D level**

Vitamin D in serum was measured by electrochemiluminescence immunoassay (ECLIA) with Roche automated immunoassay analyzer Cobas e601 using Elecsys Vitamin D Total Assay Kit (Roche Diagnostics, Mannheim, Germany) according to manufacture instruction. This method has been standardized which is traceable to the ID LC MS/MS 25 hydroxyvitamin D Reference Measurement Procedure. This LC MS/MS procedure is traceable to the National Institute of Standards and Technology Standard Reference Material 2972.

The frozen blood samples (stored at -70° C) were brought to room temperature and then homogenized through vortex mixing. Then a little portion of that plasma was transferred to a micro centrifuge tube and used for the above test in an auto analyzer according to the manufacturer’s instruction by following the above-mentioned principle. The analyzer automatically calculated the analytes concentration of each sample via a calibration curve which is an instrument specifically generated by a 2-point calibration and a master curve provided via the reagent barcode. Two level commercially available lyophilized quality control serums (PreciControl Varia 1 and 2, Roche Diagnostics, GmbH, D-68298 Mannheim, Germany) were used to check both accuracy and precision as an internal quality control material. The QC results were checked after each run and then sample analysis was started if all the QC results are within 2SD limits.

**Determination of VDR Gene Polymorphisms**

Following steps were involved to determine the VDR gene polymorphism.

**DNA Extraction from Blood sample**

Extraction of DNA from blood samples was performed by using Favorgen Blood Genomic DNA Extraction Mini Kit (Favorgen Biotech Corporation, Taiwan) according to the manufacturer’s instructions. In 200 µL of whole blood; 20 µL Proteinase K and 200 µL FABG buffer were added, vortexed and incubated at 60°C for 15 minutes. After incubation, 200 µL 96% ethanol was added and mixed properly. The sample was then transferred to the FABG column and centrifuged at 12000 RPM for 1 minute. Washing was done with
400 µL W1 buffer and 750 µL Wash buffer, respectively. Upon drying, an elution buffer was added and centrifuged at 12000 RPM to get the DNA which was stored at −20°C for further study.

**Detection of SNPs of VDR gene and Genotyping**

Four single nucleotide polymorphisms of vitamin D receptor (VDR) gene i.e., TaqI (A > G, rs731236), BsmI (C > T, rs1544410), Apal (G > T, rs11168271), and FokI (A > G, rs2227580) were genotyped by polymerase chain reaction-restriction fragment length polymorphism assay (PCR-RFLP).

The PCR reactions were performed using MasterMix (Mini16 thermal cycler) in the final volume of 25 µL of PCR mix containing 50 ng/µL DNA and 10 µM of each specific primer. PCR reaction conditions were as follows: initial denaturation at 94°C for 2 min, followed by 30 cycles of denaturation at 94°C for 30s, annealing at 60°C for FokI and BsmI, Apal and TaqI for 30s, and extension at 72°C for 90 s. The reaction was concluded with the final extension at 72°C for 300s.

After the PCR, DNA products were purified using the Favorgen PCR product purification kit according to the instructions provided in the manual. Followed by the verification of PCR products on 2% gel for FokI, Apal, TaqI, and BsmI, restriction digestion was carried out using NEB restriction enzymes (New England Biolabs, USA) for 5 min at 37°C for FokI, BsmI, and Apal and at 65°C for TaqI. Restriction fragments were analyzed by using 2% agarose gel electrophoresis. The gel was stained with Midori Green Advance DNA Stain (Nippon Genetics, Germany) and visualized under Blue Light Transilluminator. Afterward, genotypes for each participant and each of the studied VDR SNPs were determined according to the length of the obtained restriction fragments. The following table below (Table 1) shows the characterization, conditions, and primers of VDR gene polymorphism.
### Table 1
Characterization, conditions, and primers of VDR gene polymorphism

<table>
<thead>
<tr>
<th>SNP</th>
<th>Position</th>
<th>Primers (5′-3′)</th>
<th>Alleles</th>
<th>PCR products (bp)</th>
<th>Annealing Temp. (°C)</th>
<th>Restriction fragment length (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FokI</td>
<td>Exon 2</td>
<td>F: GCACTGACTCTGGCTCTGAC</td>
<td>C/T</td>
<td>265</td>
<td>37 C</td>
<td>C(F) = 265</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: ACCCTCCTGCTCTGGCT</td>
<td>(F/f)</td>
<td></td>
<td></td>
<td>T(f) = 196 + 65</td>
</tr>
<tr>
<td>BsmI</td>
<td>Intron 8</td>
<td>F: GGAGACACAGATAAGGAAATAC</td>
<td>A/G</td>
<td>301</td>
<td>37 C</td>
<td>A(B) = 301</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: CCGCAAGAAACCTCAAATAAC</td>
<td>(B/b)</td>
<td></td>
<td></td>
<td>G(b) = 250 + 180</td>
</tr>
<tr>
<td>TaqI</td>
<td>Exon 9</td>
<td>F: AGCAGGAGCAGAGTTCCAAGC</td>
<td>T/C</td>
<td>495</td>
<td>65 C</td>
<td>T(T) = 495</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: GTGAGGGGGCTGCTGAGTA</td>
<td>(T/t)</td>
<td></td>
<td></td>
<td>C(t) = 300 + 290 + 195</td>
</tr>
<tr>
<td>ApaI</td>
<td>Intron 9</td>
<td>F: AGCAGGAGCAGAGTTCCAAGC</td>
<td>A/C</td>
<td>950</td>
<td>37 C</td>
<td>A(A) = 950</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: GTGAGGGGGCTGCTGAGTA</td>
<td>(A/a)</td>
<td></td>
<td></td>
<td>C(a) = 900 + 850</td>
</tr>
</tbody>
</table>

### Statistical analysis

Students’ t-test, Pearson’s correlation analysis, and analysis of variance (ANOVA) were performed where appropriate. The chi-square test was used to detect differences in allele and genotype frequencies between groups using the wild-type genotype as reference. The OR and 95% CIs were used to measure the strength of the association between the frequencies of the VDR gene genotypes and overweight children in comparison to controls. The chi-square test was run to evaluate the significant associations between lifestyle variables and level of vitamin D in blood. The established level of significance was p < 0.05.

### Results

#### Demographic history

In this study, a total of 164 school-going children were enrolled from Dhaka city and adjacent areas of Dhaka city. Later they were categorized into four BMI groups: underweight (< 17.9), normal body weight (18-24.9), overweight (25-29.9), and obese (> 30). Participants were predominantly male (51.2%) and female (48.8%), with a mean age of 10.85 (6–13 years) years. The number of participants according to BMI includes, underweight: 43 (26.2%); normal body weight: 55 (33.54%); overweight: 43 (26.2%), and
obese: 31(18.9%). The demographic and anthropometric history of the participants, including sex, age, and BMI group, is summarized in Table 1S (Supplementary data).

**Association of body fat percentage and vitamin D levels among male and female children**

The percentage of body fat is not significantly different between the male and the female children ($p = 0.546$). However, a highly significant difference was observed in the vitamin D levels between the groups which were divided according to the BMI ($p = 0.001$) (Table 2S-Supplementary data).

**Association of vitamin D with BMI**

The mean vit-D values (mean ± SD) of the participants were 50.46 ± 19.25 in underweight, 46.37 ± 16.58 in normal body weight, 44.08 ± 15.57 in overweight, and 37.54 ± 14.39 in obese children. It has to be noted that the participants had a normal level (< 30 ng/mL) of vit-D levels in all BMI groups (Fig. 1).

A significant difference in vitamin D levels was observed among obese participants in comparison to underweight, normal body weight and overweight BMI groups. However, the vit-D level of the underweight participants was not significantly affected by the change in BMI status compared to the normal body weight ($p = 0.263$) and overweight ($p = 0.272$) participants. Similarly, the association of vit-D levels in normal body weight and overweight participants was insignificant ($p = 0.510$). Interestingly, the obese participants showed a highly significant ($p = 0.002$) effect in lowering their vit-D status compared to the underweight participants. Significant associations were also observed in comparing obese participants with the normal body weight ($p = 0.012$) and overweight ($p = 0.081$) groups, respectively. Overall, the results showed a significant effect of obesity in controlling the vit-D levels of the participants in underweight, normal body weight, and overweight groups. The results of the analysis of the association of vit-D with BMI groups are summarized in Table 3S (Supplementary data).

**Association of vitamin D levels with lifestyle**

The association of vit-D levels with the participants’ lifestyle was analyzed considering the variables for everyday sunlight exposure, sunscreen lotion use, milk consumption, and taking supplements, e.g., multivitamins, vitamin D, calcium & cod liver oil/fish oil. The number of participants with low (< 20 ng/mL), insufficient (20.1–29.9 ng/mL) and normal (> 30 ng/mL) levels of vit-D were 7, 28 and 129, respectively. The correlation of vit-D level with the duration of everyday exposure to sunlight of the participants was significant ($p = 0.003$). The results indicated that the vit-D level is significantly increased with regular exposure to sunlight. However, the remaining variables, including drinking milk and taking supplements, did not show any significance as lifestyle variables associated with the vit-D levels of the participants. The lifestyle variables and vit-D level data are summarized in Table 4S (Supplementary data).

**Association of vitamin D levels with vitamin D-sourced food**
An alternative source of the vit-D diet was considered an important variable to be associated with the vit-D status of the participants. The data presented weekly consumption of milk beverages, vit-D rich food, and vit-D rich fish and their effect on the vit-D level of the participants. A higher percentage of children (54–64%) demonstrated normal vit-D levels who took milk beverages, vit-D rich foods, and fish weekly compared to their counterparts. However, none of the diet sources have shown significant results in the vit-D levels. The summary of the findings for the association of vit-D levels with the vit-D sourced food is given in Table 5S (Supplementary data).

**Association of vitamin D levels with medical histories**

The participants' medical histories were considered to identify their association with the vit-D level. Among the clinical conditions, inflammatory bowel disorder (IBD), muscle weakness, diarrheal history, family disease history, and presence of any other disease were counted for analysis. Participants who suffered from IBD have shown a significant ($p = 0.08$) effect on their vit-D levels. All the other medical histories did not show an association with the participants' vit-D status. The medical records and the vit-D level data are summarized in Table 6S (Supplementary data).

The dark ear lobe was observed in most of the participants, ($n = 94$); and the rest have yellow ($n = 70$). Their skin burns easily ($n = 131$) rather than tanned ($n = 33$).

**Polymorphisms:**

**Evaluation of VDR FokI, Bsml, TaqI, and Apal gene polymorphisms:**

The FokI polymorphism in exon 2, BsmI polymorphism in intron 8, TaqI polymorphism in exon 9 and Apal intron 9 were determined by using specific primers. The FF genotype (homozygote of common allele) lacked a FokI restriction site and showed 2 bands at approx. 196 and 70 bp. The heterozygote of FokI displayed three fragments, designated as Ff. Subjects homozygous for the BsmI restriction site are designated bb and show two fragments at approx. 650 and 200 bp while homozygous for the absence of the site are designated BB and give one band at 850 bp and the heterozygote type gives three bands. Similarly, the heterozygote of TaqI and Apal displayed three fragments designated as Tt and Aa, subject’s homozygous for TaqI are designated tt and aa and showed two fragments but for Apal any two bands were not found.

Allelic comparison of four polymorphic sites of VDR illustrated that Fok-I (f), Apa-I (a), TaqI (t) and BsmI (b) recessive alleles were significantly different in obese children which is shown in the following table (Table 2).
Table 2
Comparison of genotype and allele frequencies for the FokI, TaqI, BsmI, ApaI VDR polymorphisms between control and obese children.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Frequency</th>
<th>p value</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FF</td>
<td>Ff</td>
<td>ff</td>
</tr>
<tr>
<td>FokI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obese</td>
<td>10.34%</td>
<td>89.65%</td>
<td>0%</td>
</tr>
<tr>
<td>Control</td>
<td>43.33%</td>
<td>53.33%</td>
<td>3.45%</td>
</tr>
<tr>
<td>BsmI</td>
<td>BB</td>
<td>Bb</td>
<td>bb</td>
</tr>
<tr>
<td>Obese</td>
<td>24.14%</td>
<td>58.62%</td>
<td>17.24%</td>
</tr>
<tr>
<td>Control</td>
<td>20%</td>
<td>73.33%</td>
<td>6.67%</td>
</tr>
<tr>
<td>ApaI</td>
<td>AA</td>
<td>Aa</td>
<td>aa</td>
</tr>
<tr>
<td>Obese</td>
<td>31.03%</td>
<td>68.96%</td>
<td>0%</td>
</tr>
<tr>
<td>Control</td>
<td>40%</td>
<td>60%</td>
<td>0%</td>
</tr>
<tr>
<td>TaqI</td>
<td>TT</td>
<td>Tt</td>
<td>tt</td>
</tr>
<tr>
<td>Obese</td>
<td>72.41%</td>
<td>0%</td>
<td>27.59%</td>
</tr>
<tr>
<td>Control</td>
<td>33.33%</td>
<td>36.67%</td>
<td>30%</td>
</tr>
</tbody>
</table>

Control (Normal BMI) n=30; Obese n=29
*Significant at p<0.05.

For FokI as indicated in Table 2, 44.82% of obese children carry f allele, while only 25.86% of control individuals hold that allele. The results recorded for BsmI was 37.93% for both obese and control children carrying b allele. For TaqI and ApaI, as indicated in Table 2, 34.49% and 31.03% of obese children carry f allele, respectively, while only 27.58% and 46.55% of control individuals hold that allele. Based on the significant allelic relationship, further analysis on genotypes was encouraged. Further analysis has been conducted based on VDR gene polymorphisms and Vitamin D serum levels to explore the possible relationships between different genotypes and serum levels of vitamin D in patients and control (Table 3). As illustrated in Table 3, none of the singular VDR gene polymorphic sites showed a relationship with vitamin D serum levels.
Table 3
Association of Vitamin D3 level and the VDR polymorphism in obese children

<table>
<thead>
<tr>
<th>Vitamin D level</th>
<th>Sufficient</th>
<th>Insufficient</th>
<th>Deficient</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FokI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FF</td>
<td>5.26%</td>
<td>78.26%</td>
<td>0%</td>
<td>0.24</td>
</tr>
<tr>
<td>Ff</td>
<td>94.73%</td>
<td>21.74%</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>ff</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td><strong>BsmI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BB</td>
<td>20%</td>
<td>33.33%</td>
<td>33.33%</td>
<td>0.96</td>
</tr>
<tr>
<td>Bb</td>
<td>75%</td>
<td>33.33%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>bb</td>
<td>5%</td>
<td>33.33%</td>
<td>66.67%</td>
<td></td>
</tr>
<tr>
<td><strong>TaqI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>65%</td>
<td>83.33%</td>
<td>100%</td>
<td>0.009**</td>
</tr>
<tr>
<td>Tt</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>tt</td>
<td>35%</td>
<td>16.67%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td><strong>ApaI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>42.85%</td>
<td>0%</td>
<td>0%</td>
<td>0.026*</td>
</tr>
<tr>
<td>Aa</td>
<td>57.14%</td>
<td>100%</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>aa</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td></td>
</tr>
</tbody>
</table>

Obese n = 29
Significant at *p < 0.05; and ** p < 0.01.

Hundred percent of obese children showed homogenous alleli of TaqI (TT) VDR gene (p = 0.009). On the other hand, heterogenous alleli of ApaI (Aa) of VDR gene was observed in 100% obese children (p = 0.026). Moreover, bb mutation is observed in BsmI of VDR among 67% of obese children. Homogenous alleli represents the same inherited genetic marker from each biological parent. In the heterozygous alleli, a pair of alleles inherited from each parent with a different version. In a heterozygous genotype, the dominant trait is expressed. The following figures (Figs. 2 and 3) showed the gel electrophoresis of VDR gene polymorphism detected in control group and obese group of children.

For FokI as indicated in Table 3 44.82% of obese children carry f allele, whereas 3.33% of overweight children carry f allele, while only 25.86% of control individuals hold that allele. The same results were also recorded for Bsml as 37.93% both of obese and control child carry b allele but in case of overweight children it was 48.28%. For TaqI and ApaI as indicated in Table 7S (Supplementary data), 34.49% and 31.03% of obese children carry t and a allele, respectively, whereas in overweight children it is 6.66% and
8.62% while only 27.58% and 46.55% of control individuals hold that allele. Based on the significant allelic relationship, further analysis on genotypes was encouraged. Further analysis has been conducted based on VDR gene polymorphisms and Vitamin D serum levels to explore the possible relationships between different genotypes and serum levels of vitamin D in obese children, overweight children, and control (Table 7S-8S-Supplementary data). As illustrated, none of our singular VDR gene polymorphic sites showed any kind of relationship with vitamin D serum levels significantly that much.

Discussion

A wide array of studies has identified several factors causing vitamin D deficiency and thus, leading to childhood obesity such as genetic factors, environmental factors, lifestyle, dietary and supplementation intake, comorbid diseases, etc.13,17–19. In the current study, we have investigated the association of vitamin D levels and VDR gene polymorphism with childhood obesity. To determine the connection between obesity and levels of vitamin D, children's BMI, lifestyle, and dietary intake were taken into consideration and studied. Further to reveal the association between obesity and VDR gene polymorphisms four single nucleotide polymorphisms of the VDR gene including ApaI, FokI, TaqI, and BsmI were selected and investigated.

1. Association Of Vitamin D Levels And Bmi

Defining and determining obesity in children is challenging since there is no standard threshold set for them like adults. Therefore, obesity diagnosis in children is normally determined by BMI calculation20. Studies have reported a major impact of BMI on vitamin D levels. An inverse relationship between vitamin D deficiency and BMI has been established by multiple studies conducted previously21.

In this study, a total of 164 school-going Bangladeshi children between 6–13 years old (with a mean age of 10.85 years) were included and of them, male participants were predominant (51.2%) and the female participants were 48.8%. Participants were categorized into 4 groups according to their BMI including underweight (< 17.9), normal body weight (18-24.9), overweight (25-29.9), and obese (> 30). BMI measurement confirmed that 31 (18.9%) and 35 (21.3%) children were obese and overweight, respectively. The findings of serum vitamin D level analysis showed that the lowest vitamin D level (37.54 ± 14.39 ng/mL) was found in obese participants, whereas, the highest vitamin D level (50.46 ± 19.25) was determined in underweight children. Overweight children had a lower vitamin D concentration (44.08 ± 15.57) than normal-weight children (46.37 ± 16.58). From the ANOVA test, it is noteworthy to mention that a highly significant (F = 3.710; p = 0.013) correlation between low levels of vitamin D with obesity in children was found compared to underweight children; normal body weight, and overweight children. Furthermore, significant (p = 0.012, p = 0.081) associations of vitamin D deficiency with obese children were observed compared to under-weight (p = 0.002), normal body weight (p = 0.012) and overweight (p = 0.081), respectively. A previously conducted study to reveal the association of 25(OH)D concentration with BMI suggested that each 10% increase in body weight resulted in a 4.2% reduction in
vitamin D concentration\textsuperscript{22}. In 278 children and adolescents, a homeostatic model analysis revealed a significant correlation between BMI and 25(OH)D deficiency (p < 0.001)\textsuperscript{23}. A study on 9–13 years old Spanish children found an association between BMI and abdominal obesity and vit-D insufficiency\textsuperscript{24}.

2. Association Of Vitamin D Levels With Lifestyle

A wide range of research suggests the association of vitamin D levels with lifestyle\textsuperscript{25}. Since vitamin D is synthesized on exposure to sunlight spending much time indoors and using sun protection are reported as two vital causes of vitamin D deficiency\textsuperscript{26}. In our study among 164 children 7 participants had low levels of vitamin D (\(> 20\) ng/mL), 28 participants had insufficient levels of vitamin D (20.1–29.9 ng/mL) and 129 had normal levels of vit-D (< 30 ng/mL). Our findings showed a significant (p = 0.003) correlation was found between sunlight exposure time and vitamin D levels. In our study, increased/sufficient/normal vit-D levels were determined in children who had regular sunlight exposure (~ 1.4 hr) and spent more time in outdoor activities (~ 1.3 hr). Our study findings are scientifically supported by the research performed by Das et al., on Bangladeshi urban and rural 12–24 months old children\textsuperscript{27}. Similarly, a large multiethnic cohort study on 6-year-old children in the Netherland revealed that 30% of children were found with vit-D deficiency and the causative factors identified were spending much time indoors for example more television watching, less biking to school, and playing less outside\textsuperscript{26}. The association of vitamin D status with physical activity, participants’ habits, and sedentary life was studied on 5–11 years old school-going children (a total of 200 participants) in Northern Ireland, and the study outcomes demonstrated the higher prevalence of lower vit-D levels in children who spent much time indoor and sedentary life. In contrast, vit-D sufficient children had spent significantly higher hours outdoors and less inactive time\textsuperscript{28}. A higher prevalence of vit-D deficiency was found in US children (aged 1–21 years) with a more indoor and sedentary lifestyle\textsuperscript{29}. A cross-sectional study on Belgian children to determine the association of dietary and lifestyle-induced health effects with vit-D status demonstrated the weekly number of hours playing outdoors as a potential determinant of vitamin D status\textsuperscript{24}. Similar findings were also reported by another research group where 5–11 years old Saudi Arabian school-going children were studied to find the link between vit-D levels and lifestyle (Exposure to sunlight and more physical activity). Children with no sunlight exposure had significantly lower vit-D levels which were increased with increasing exposure time to sunlight\textsuperscript{24}. A cross-sectional study was conducted on a total of 150 urban and rural Bangladeshi children where children with inadequate sunlight exposure had 2.5 times higher vit-D deficiency compared to children with adequate sunlight exposure\textsuperscript{30}. Another study was conducted on children of the South-East region of Bangladesh and less sun exposure was identified as one of the risk factors for hypovitaminosis D among the children\textsuperscript{31}. Similar study findings have been reported by other research groups globally\textsuperscript{32–34}.

3. Association Of Vitamin D Levels With Dietary, And Supplementation Intake
Various research suggests the association of vitamin D levels with dietary, and supplementation intake\textsuperscript{25,26}. In our study, although not statistically significant, a higher percentage of children who had milk, milk beverages, vit-D rich foods, and vit-D rich fish weekly showed sufficient vit-D levels. A previous study reported a significant association between higher dietary vit-D (\(p = 0.021\)) and vit-D supplements (\(p = 0.028\)) daily intake and vit-D sufficient participants\textsuperscript{28}. Some studies revealed that dietary intake of certain vit-D-rich foods has a significant influence on vit-D deficiency reduction\textsuperscript{19}. Another research demonstrated that regular consumption of vit-D-rich foods, vit-D fortified foods, and vit-D supplementation can contribute significantly to improving vit-D status in children\textsuperscript{28}. Among Canadian children who consumed vit-D fortified milk daily (77\%) and vit-D-containing supplements (9\%) were reported to have sufficient levels of vit-D\textsuperscript{35}. Giving critical importance to vit-D levels in children the International Health Organizations, namely the Institute of Medicine (IOM) has placed guidelines and recommendations to surmount widespread vit-D deficiency challenges. Also, the website of the US Department of Agriculture's Nutrient Database has a list of foods and their vit-D content for general use\textsuperscript{36}.

4. Association Of Vitamin D Levels With Medical Histories

Epidemiological studies have demonstrated an association of vit-D deficiency with various chronic diseases including metabolic disorders, cancer, cardiovascular diseases, diabetes, neuropsychiatric disorders, autoimmune diseases, and infectious diseases\textsuperscript{37}. Thus, in our study, clinical conditions such as inflammatory bowel disease (IBD), diarrheal history, autoimmune diseases such as rheumatoid arthritis, osteoarthritis, muscle weakness, kidney disease, etc., were considered, and data were collected and analyzed. Among all these a significant (\(p = 0.08\)) correlation was found between children suffering from IBD and vit-D deficiency. The findings of our study are in line with the results of the previous studies that reported a high prevalence of vit-D deficiency in children with IBD. For instance, 77 (80.2\%) Korean children and adolescents out of 96 were identified with vit-D deficiency and a positive correlation was found between vit-D deficiency and IBD\textsuperscript{38}. Similarly, another study reported that hypovitaminosis D was most prevalent (44\%; \(P = 0.05\)) in children and adolescents suffering from IDB (Crohn's disease)\textsuperscript{39}. In a randomized controlled trial on 120 children with IBD and vit-D deficiency supplementation of vit-D significantly improved the condition compared to the placebo group\textsuperscript{40}. Suboptimal vit-D concentration was most prevalent in children with Crohn's disease (69.1\% followed by ulcerative colitis (46.4\%) in a previous study conducted by Jasielska and Grzybowska-Chlebowczyk\textsuperscript{41}. In addition to that, children with IBD are more likely to be at an increased risk of developing vit-D deficiency due to malabsorption of bile salts, impaired absorption of nutrients, restricted dietary intake, and physician's advice to avoid sunlight exposure while taking immunosuppressants\textsuperscript{42}.

Vitamin D signaling plays an important role in the maintenance of epithelial barrier integrity by regulating epithelial cell gap junction proteins and protects against infection and inflammation by enhancing its resistance to irritants, increasing epithelial cell repair, and reducing epithelial cell apoptosis. On the
contrary, hypovitaminosis D disrupts gut barrier integrity and immune functions, increasing translocation of the gut microbiome and dysbiosis, and thus, leading to initiation and progression of IBD\textsuperscript{42,43}.

5. The association of vitamin D levels and vitamin D receptor gene polymorphism with obesity and overweight in children

Obesity and overweight have become an epidemic problem in different areas, and its rate is increasing across the world. Since vitamin D deficiency is still a silent problem, we must take the necessary precautions to avoid it.\textsuperscript{44} Different studies in different countries identified an association between obesity and low vitamin D level\textsuperscript{10}. In 2015, researchers in China who were studying obese people discovered a link between vitamin D deficiency and obesity\textsuperscript{45}.

The VDR gene is located at the 12q13 chromosome and many polymorphisms are indicated at this region. The VDR genes are the FokI, BsmI, TaqI, and ApaI\textsuperscript{46}. Among these genes polymorphisms have an important role in the function of the VDR receptor. The FokI polymorphism which is located in exon 2 is linked with a second methionine start site, forming a shorter protein receptor\textsuperscript{47}. If we compare, we would see that this receptor has greater transcriptional activity than the wild type receptor. The BsmI polymorphism can be linked to a variable length polyadenylate sequence within the 3′-untranslated region. Bsml genotype frequencies for the obese child and control groups had a statistically significant difference with a P value of 0.02. The BB and Bb genotypes were significantly associated with obesity.

The study showed the decreased level of VDR mRNA with the VDR B allele compared to those not bearing the B allele\textsuperscript{48}. A previous study on a French population, found an association between Bsml genotypes and obesity. For French Caucasians with type 2 diabetes mellitus, VDR is not a significant gene. However, in people with early-onset type 2 diabetes mellitus, polymorphisms in the VDR gene are linked to an increased risk of obesity. The pathophysiological processes behind these correlations are yet unknown, however they may be connected to either a direct role for vitamin D in the development and metabolism of adipocytes or a secondary role for vitamin D in modulating insulin production. They also found that a bb genotype is more susceptible to obesity than BB and Bb genotypes\textsuperscript{49}. To the contrary, in our study there was no contribution of the VDR polymorphism FokI, TaqI and APaI to obese children with a P value of 0.47, 0.75 and 0.15, respectively. In contrast the polymorphic alleles (TT) of ApaI seems to be a risk factor for asthma in children with normal weight, while that of Bsml seems to be a risk factor for asthma in overweight conditions. In a study on Brazilian school children, it has been found that children with asthma who are eutrophic have a greater frequency of the TT allele for the ApaI gene, while children with asthma who are overweight have a higher frequency of the TT allele for the Bsml gene. That means weight might be a factor\textsuperscript{10}. In a population-based case–control comprehensive study, the severity of vitamin D gene variants including FokI, Bsml, TaqI, and Apal has been accomplished in the Iranian population. Their results indicated that the VDR Apal polymorphism and obesity susceptibility, whereas the Apal A allele and AA genotype, were related to obesity phenotypes. The higher serum levels of FBS and BMI in genotype AA carriers provided clear evidence of the associations between the VDR gene
polymorphisms and the anthropometric and biochemical characteristics of obesity \(^5\). In our recent study, positive findings in polymorphisms of vitamin D-related genes were obtained. Among the four kinds of VDR SNPs, the FokI/Ff allele, Bsml/bb allele, TaqI/Tt allele, and ApaI/Aa genotypes showed an association with vitamin D3 level in obese children. On the other hand, a study done on Indian women did not confirm this association \(^50\). According to TaqI genotypes, in 2011, a study on a Vietnamese population confirmed the association between TaqI genotypes and development of obesity \(^51\). Also, a recent study confirmed this association on an Egyptian population. Here a recent cross-over experiment has been carried out that was randomized, double-blinded, and controlled for placebo, 39 type 1 diabetes patients got 4000 IU of cholecalciferol per day for three months before receiving placebo or the opposite treatment. As a result, the vitamin D status also improved, and the regulatory T cells (Treg) showed a distinct response to vitamin D after three months of treatment based on VDR SNPs. Additionally, under vitamin D administration, this experiment demonstrated an improvement in glycemic parameters. The number of Treg cells could be increased in patients with the genotypes aa, TT, and bb. In case of our results, we only found that tt genotype is not associated with obesity may be due to the genetic variation in Egyptian region and Asian region \(^52\). Regarding our study limitations, it can be said that this study was conducted on school going obese children in Bangladesh, which represents a small size sample, so a large sample study is recommended to confirm the association of VDR polymorphism and obesity. We have also investigated the VDR polymorphism study in overweight children as well. However, we found that there was no significant association of VDR polymorphism with Vitamin D serum level in overweight children of Bangladesh whereas a study that has been done in Brazil in overweight children found out that Bsml might be a risk factor in overweight children to develop asthma disease \(^10\). It has been shown that children and adolescents who are overweight or obese have a significant prevalence of vitamin D deficiency, which increases as obesity becomes more severe. A study highlights that the necessity for larger cholecalciferol dosages to attain serum calcifediol objectives in overweight and obese children and adolescents as well as the most recent recommendations for treating vitamin D deficiency, while in our study it does not reflect any significance which might be variable due to genetic variation, ethnicity, area etc. \(^20\). In children, low serum 25(OH)-D is positively correlated with obesity or high BMI. Genetic factors at least partially affect the levels of vitamin D in the blood. The adipogenesis process and the level of inflammation in adipocytes and adipose tissue are both significantly influenced by vitamin D \(^53\). The GGT haplotype and the Bsml, Apal, and TaqI wild variations of the VDR gene were connected to reduced vitamin D levels, indicating that VDR gene polymorphisms may increase a subpopulation of children's susceptibility to vitamin D insufficiency. It has been claimed that, this is the first report in South Brazil relating VDR gene polymorphisms and haplotypes to 25(OH)D levels in healthy children and adolescent girls from the general population. Other studies that focused on a particular condition, such as the overweight Insulin Resistance Atherosclerosis family study, in which only the Bsml SNP was examined, and the Metabolites in Multiple Sclerosis study, which covered the Apal and TaqI SNPs, did not discover a relationship between the VDR polymorphism and 25(OH)D levels. These investigations, however, involved adult populations with disease, whereas the patients in our results were
younger and healthier. BsmI, ApaI, and TaqI SNPs of the VDR gene were not linked to 25(OH)D levels in investigations including older, overweight patients.\textsuperscript{54}

**Conclusion**

This study is a pioneering investigation demonstrating the association of serum vit-D levels and VDR gene polymorphisms with childhood obesity in Bangladeshi School going children. The study findings demonstrated significantly lower levels of vit-D in obese children compared to the control group. Children who had exposure to sunlight during the daytime, for around one hour, had higher serum levels of vit-D and thus, suggesting this may help to increase vitamin D levels in the body. VDR gene polymorphism, the variations in DNA sequence, have an important role in the function of vit-D receptors. Allelic comparison of four polymorphic sites of VDR illustrated that Fok-I (f), Apa-I (a), TaqI (t), and BsmI (b) recessive alleles were different in obese children compared to control individuals. A significant difference was observed in the BsmI alleles (BB, Bb, and bb) ($p = 0.02$) in obese children compared to the control group. Our study outcomes indicate that vit-D deficiency and VDR-BsmI polymorphism are the risk factors for childhood obesity. Daily exposure to sunlight at least for an hour and therapeutic intervention in obese children might help in alleviating obesity-related clinical and psychosocial consequences.

**Future recommendations**

1. Further study needs to be conducted including many urban obese children who have limited exposure to sunlight.
2. Regular exposure of obese children to sunlight is recommended as sunlight (UVB radiation) activates vit-D synthesis and increases vit-D levels in the body.
3. This research has a great impact on public health. Thus, fulfillment of vit-D requirements and implementation of adequate lifestyle interventions are recommended to prevent obesity and vit-D deficiency-related health problems in children.

**Declarations**

**Data Availability**

The datasets used and/or analyzed to construct figures or tables during the current study are available from the corresponding author on reasonable request.

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**Contributions**
The study is conceptualized and designed by SN; Samples were collected by SN and RR; Method was developed by MAH, Analysis of samples were performed by MAH, RR, and AKR; Data was analyzed and interpreted by SS, AA; Manuscript was written by RA, AA, SS, and SN; Manuscript was revised by RA, AA, and SN.

**Ethics Declarations**

**Competing Interests**

Authors declare no competing interest.

**References**


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Figures
Figure 1

Association of Vitamin D with Different Body Mass Index (BMI)

Here, 1=Underweight; 2=Normal weight; 3=Overweight; 4=Obese

***, **, and * represent the difference of body weight with obesity are significant at 1%, 5%, and 10% levels, respectively.
Figure 2

Figure 3


Supplementary Files

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- 15.03.2023SupplementaryData.zip