Vitamin D and its Association with FokI VDR Polymorphism in Patients with Asthma-COPD Overlap


Abstract

Background:
Asthma-COPD overlap is a new phenotype in respiratory ailments. It has been shown that this group of patients might possess vitamin D deficiency. The association of vitamin D receptor (VDR) gene polymorphism with serum vitamin D status in ACO patients has not been investigated yet.

Objective:
To assess the association of VDR gene polymorphism (FokI) with serum vitamin D status in patients with Asthma-COPD overlap.

Methods:
This cross-sectional study was carried out in the Department of Physiology of Bangabandhu Sheikh Mujib Medical University, Dhaka, from January 2018 to July 2018 on 23 (twenty-three) patients (aged ≥40 years) with ACO. For comparison, 24 (twenty-four) apparently healthy age, smoking duration and BMI matched subjects were selected. For all participants single nucleotide polymorphism of VDR gene FokI (rs10735810) was done by DNA extraction, polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). Vitamin D was measured by using automated analyzer: ARCHITECT Plus ci4100. The results were expressed as mean with standard error (mean±SEM) and frequency distribution. The data were statistically analyzed by Graphpad prism (Version 7) using independent sample ‘t’ test, two sample proportion test, Fisher exact test and ANOVA test. In the interpretation of results, ≤0.05 level of probability (p) was accepted as significant.

Results:
The mean±SEM of serum 25(OH)D were 16.37±0.78 and 18.46±1.01 ng/ml in control and study groups, respectively. The frequency distribution of FokI genotype was 86.95% (FF), 8.69% (Ff), 4.35% (ff) and 91.66% (FF), 4.17% (Ff), 4.17% (ff) in ACO patients and healthy subjects, respectively. When FokI VDR SNP was analyzed with serum vitamin D status in patients with ACO, statistically no association was seen.

Conclusions:
FokI VDR SNP is not associated with serum vitamin D status in patients with ACO.

Keywords: Vitamin D, Asthma-COPD overlap, Vitamin D receptor gene, single nucleotide polymorphism, FokI (rs10735810)

Introduction:
Vitamin D is a fat-soluble steroid hormone, well known for its classical role in bone mineralization and calcium homeostasis. Deficiency of this vitamin D might cause rickets, osteomalacia, low bone mass, osteoporosis as well as fractures. It has been observed that non classical role of Vitamin D may possess health benefits, such as prevention of autoimmune disease, cardiovascular disorders, hypertension and influenza. Higher serum vitamin D concentration has been associated with better lung function as measured by FEV1 in a large cross-sectional study of the US population.
significant proportion of young COPD patients were with insufficient (20 to 29 ng/ml) serum 25(OH)D. Furthermore, it has been suggested that, lower vitamin D status in COPD might be due to diminished production of pre-vitamin D₃ associated with skin aging caused by smoking and limited UVB radiation exposure⁵,⁶. Statistically significant association between vitamin D deficiency and asthma has been reported⁷,⁸. This vitamin’s deficiency was also found to increase the risk of severe asthma exacerbation and need for emergency department evaluation or hospitalization⁹. In a very recent study, Vitamin D supplementation was shown to reduce the rate of asthma exacerbations requiring treatment with systemic corticosteroids. However, as far as it has been searched, association of vitamin D status with ACO has only been observed by Odler et al. (2015) along with other airway disorders (COPD and asthma). They reported 60% of ACO, 76% of COPD and 36% of asthmatic patients had vitamin D deficiency⁴.

For any cellular action, activated vitamin D₃ binds to its specific nuclear vitamin D receptor (VDR) in the target tissue⁴. VDR gene is located on 12q13.11 possessing 11 exons with a length of 5.6 kb. The VDR gene has more than 470 single nucleotide polymorphisms (SNPs), a number of which modulate the uptake of 1, 25(OH)₂D₃. Among them most commonly studied VDR polymorphisms include Apal (rs7975232), BsmI (rs1544410), Taql (rs731236) and FokI (rs10735810)¹⁰,¹⁶,¹⁷

In the perspective of respiratory ailments only a few numbers of studies reported association of VDR SNPs in COPD and in asthma. No statistically significant relationship was found between Taql and FokI VDR gene and susceptibility to tuberculosis. When VDR gene polymorphisms were analyzed with respect to plasma vitamin D levels in TB patients, a significant association was seen. In this study we present the frequency of FokI polymorphism in healthy subjects as well as ACO patients of Bangladesh and its association with vitamin D.

**Methods:**
This cross-sectional study was conducted from January 2018 to July 2018 in the Department of Physiology and Center for Advanced Biomedical Research, Bangabandhu Sheikh Mujib University (BSMMU). The protocol was approved by the institutional review board of BSMMU. 23 pulmonologist diagnosed male patients of ACO enrolled by consecutive sampling from outpatient Department of NIDCH. For comparison, 24 age, BMI, duration of smoking matched apparently healthy males were selected from community. A written informed consent was taken from all the participants after detailing of study procedure. With all aseptic precautions 5 ml venous blood was drawn from ante-cubital vein by a disposable plastic syringe for analysis of vitamin D receptor gene polymorphism and for estimation of serum 25(OH)D. 4 ml in plain tube and 1 ml in EDTA tube was preserved for estimation of Serum 25(OH)D and for analysis of vitamin D receptor gene polymorphism. DNA extraction was done by Thermo scientific Genejet genomic DNA purification kit, Lithuania. Estimation of serum 25-hydroxycholecalciferol (Vitamin D₃) was measured by using automated analyzer: ARCHITECT Plus ci4100.

**FokI Polymorphism:**
VDR gene amplification was performed by PCR in 25μl reaction mixtures containing primers for FokI polymorphism. The PCR reaction mixture consisted of 4 μl of genomic DNA, PCR Mastermix 10 μl, 1 μl of forward and reverse primers, (Thermo Scientific) and nuclease free water 9μl. The PCR cycling conditions were initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 30 sec, 58°C for 30 sec and 72°C for 1 min and one final cycle of extension at 72°C for 7 minutes. The primers for FokI polymorphism were 5’-GATGCCAGCTGCGCCTGGCACAGT-3’ and 5’-ATGGAAACACCTGTCTCTCTCCCT-3’. The PCR product (272 bp) was digested with 1.0 unit of FokI restriction enzyme (Thermo Scientific FastDigest enzyme) at 37°C for 5 minutes. Then 10 μl digested reaction mixture was loaded into 2% agarose gel containing ethidium bromide. Subjects with 272bp designated as FF, 2 bands of 198 bp and 74 bp designated as Ff and three bands of 272, 198, and 74 bp were labeled as Ff.

**Statistical analysis:**
The results were expressed as mean with standard error (mean±SEM) and frequency distribution. The data were statistically analyzed by Graphpad prism (Version 7) using independent sample ‘t’ test, two sample proportion test, Fisher exact test
been observed that non classical role of Vitamin D better lung function as measured by FEV1 in a hypertension and influenza. Higher serum may possess health benefits, such as prevention of polymorphisms include ApaI (rs7975232), BsmI Among them most commonly studied VDR susceptibility to tuberculosis. When VDR gene between TaqI and FokI VDR gene and 12q13.11 possessing 11 exons with a length of 5.6

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This cross-sectional study was conducted from January to March 2023. The study was performed in the Pulmonary Medicine Outpatient Department of the University Hospital, Dhaka, Bangladesh. All patients were recruited from the Pulmonary Medicine Outpatient Department of the University Hospital, Dhaka, Bangladesh. The protocol was approved by the Institutional Review Board of the University Hospital, Dhaka, Bangladesh.

Participants were selected from community. A total of 47 participants were included in the study. The participants were divided into two groups: group A (apparently healthy subjects) and group B (patients with ACO). All participants provided written informed consent. The mean±SEM of serum 25(OH)D were 16.37±0.78 and 18.46±1.01 ng/ml in control and study groups patients, respectively. (Table-I)

### Table-I: Serum 25(OH)D level in two groups (n=47)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>A (n=24)</th>
<th>B (n=23)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D ng/ml</td>
<td>19.5±2.42</td>
<td>18.9±2.72</td>
<td>0.11</td>
</tr>
<tr>
<td>mean±SEM</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Statistical analysis was done by independent sample 't' test.

n=number of subjects.

A=Apparently healthy subjects.

B=Patients with ACO.

25(OH)D=Serum 25- Hydroxycholecalciferol

### Table-II: Association of FokI genotype with Serum vitamin D3 status in two groups (n=47)

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>A (n=24)</th>
<th>B (n=23)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FF</td>
<td>19</td>
<td>3</td>
<td>0.31</td>
</tr>
<tr>
<td>D3 deficient</td>
<td>12</td>
<td>8</td>
<td>1.00</td>
</tr>
<tr>
<td>Ff+ff</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Statistical analysis was done by Fisher exact test.

n=number of subjects.

A=Apparently healthy subjects.

B=Patients with Asthma-COPD overlap

### Serum vitamin D3 status in different genotypes of FokI

All genotype frequencies of FokI vitamin D receptor gene were analysed with 25(OH)D concentration. In the present study, mean±SEM of 25(OH)D were 17.29±0.69, 18.67±2.31 and 17.60±4.60 in FF, Ff and ff genotypes of FokI, respectively. However, the differences of vitamin D level among the subjects with 3 genotypes of FokI was statistically non significant. (Table-III)

### Table-III: Serum vitamin D3 status among subjects with different genotypes of FokI (n=47)

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Serum 25(OH)D (ng/ml)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FF (n=42)</td>
<td>17.29±0.69</td>
<td></td>
</tr>
<tr>
<td>Ff (n=3)</td>
<td>18.67±2.31</td>
<td>0.88</td>
</tr>
<tr>
<td>ff(n=2)</td>
<td>17.60±4.60</td>
<td></td>
</tr>
</tbody>
</table>

Statistical analysis was done by ANOVA.

n=number of subjects
Vitamin D and its Association with FokI VDR Polymorphism

Discussion:
In our study, all subjects in both the healthy control as well as ACO patients had serum 25(OH)D deficiency (<30 ng/ml) (vitamin D council 2017). Similar deficiency was also reported in apparently healthy population of Bangladesh22-23 and other countries24 as well as in ACO patients11. Indoor staying from 10 AM to 2PM 25 and darker skin type25,26 of our study subjects might be obstacle for conversion of 7-dehydrocholesterol to pre vitamin D₃ in the skin and could be responsible for this vitamin deficiency. As both of our healthy subjects as well as ACO patients were smoker and the smoking habit individually has a deleterious influence on serum 25(OH)D24, so this life style could be a causative factor for our findings. No significant difference was found in the mean serum 25(OH)D level between our control and ACO patients. Similar finding was reported by Odler et al. (2015) in Hungarian ACO population. Another finding of our study was no association of frequency of different genotypes of FokI SNP with serum vitamin D₃ status both in healthy subjects as well as ACO population. Similar finding was reported in Indian healthy population by Bhanushali et al. (2009) though their categorization of D₃ status was different from us 27. On the other hand, significant (p<0.001) association of frequency of FokI genotype with D₃ status was reported in Pakistani healthy women28. No significant difference was observed in the mean serum D₃ level among our samples bearing different genotypes of FokI. No similar observation was available, for comparison. No mechanism of our research findings could be proposed as no previous study on VDR in ACO was found to compare our result.

Conclusion
Bangladeshi healthy population as well as ACO patients might have D₃ deficiency and FokI VDR SNP was not associated with D₃ deficiency. Too small sample size was the limitation of this study. To overcome the limitation recommendation is to observe similar type of study with large sample size.

References:
1. Mahmood S, Rahman M, Biswas SK, Saqueeb SN, Zaman S, Manirujaman M, et al. Vitamin D and Parathyroid Hormone Status in Female...


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