Vitamin D and it's Association with FokI VDR Polymorphism in Patients with Asthma-COPD Overlap

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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_3 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_3 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[1]. It has been observed that non classical role of Vitamin D may possess health benefits, such as prevention of autoimmune disease[2], cardiovascular disorders[3,4], hypertension[5] and influenza[6]. 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[7]. Recently, a group of researchers reported that a

significant proportion of young COPD patients were with insufficient (20 to 29 ng/ml) serum 25(OH)D[8]. Furthermore, it has been suggested that, lower vitamin D status in COPD might be due to diminished production of pre-vitamin D_3 associated with skin aging caused by smoking and limited UVB radiation exposure[3,9]. Statistically significant association between vitamin D deficiency and asthma has been reported[10]. This vitamin's deficiency was also found to increase the risk of severe asthma exacerbation and need for emergency department evaluation or hospitalization[11]. In a very recent study, Vitamin D supplementation was shown to reduce the rate of asthma exacerbations requiring treatment with systemic corticosteroids[12]. 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 deficiency[13].

For any cellular action, activated vitamin D_3 binds to its specific nuclear vitamin D receptor (VDR) in the target tissue[14]. VDR gene is located on 12q13.11 possessing 11 exons with a length of 5.6 kb[15]. The VDR gene has more than 470 single nucleotide polymorphisms (SNPs), a number of which modulate the uptake of 1, 25(OH)$2D_3$[16]. Among them most commonly studied VDR polymorphisms include ApaI (rs7975232), BsmI (rs1544410), TaqI (rs731236) and FokI (rs10735810)[16,17]

In the perspective of respiratory ailments only a few numbers of studies reported association of VDR SNPs in COPD[18] and in asthma[19,20]. No statistically significant relationship was found between TaqI and FokI VDR gene and susceptibility to tuberculosis[17]. When VDR gene polymorphisms were analyzed with respect to plasma vitamin D levels in TB patients, a significant association was seen[17]. 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 Medical 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, Lithunia.Estimation of serum 25-hydroxycholecalciferol (Vitamin $D)_3$ 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[21]. 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' -GATGCCAGCTGGCCCTGGCACTG-3′ and 5′ -ATGGAAACACCTTGCTTCTTCTCCCTC-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

and ANOVA test. In the interpretation of results, ≤ 0.05 level of probability (p) was accepted as significant.

Results:

In the present study, 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)

Parameter	A (n=24)	B (n=23)	p-value
25(OH)D ng/ml mean±SEM	16.37±0.78 (9.5-24.2)	18.46±1.01 (8.9-27.2)	0.11

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

Association of FokI genotype with Serum vitamin D_3 status

Association of vitamin D level with FokI SNP was studied in 47 subjects who were divided into two categories as vitamin D deficient and insufficient. Out of 24 apparently healthy subjects 20 were deficient and 4 had insufficient level. Of the 20 D_3 deficient healthy subjects 19 were with FF and 1 was with non FF (Ff/ff). Of the 4 D_3 insufficient healthy subjects 3 were with FF and 1 was with non FF (Ff/ff). In case of 23 study subjects 14 were deficient and 9 had insufficient level. Of the 14 D_3 deficient subjects 12 were with FF and 2 was with non FF (Ff/ff). Of the 9 D_3 insufficient subjects 8 were with FF and 1 was with non FF (Ff/ff). However, the difference of frequency in different genotypes of FokI was statistically non-significant with serum 25 (OH)D, both in healthy as well as in ACO patients. (Table-II)

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

Genotypes	A (n=24)			B (n=23)		
	D3 deficient	D_3 insufficient	p value	D_3 deficient	D_3 insufficient	p-value
FF	19	3		12	8	1.00
Ff+ff	1	1	0.31	2	1	

Statistical analysis was done by Fisher exact test.
n=number of subjects.
A=Apparently healthy subjects.
B=Patients with Asthma-COPD overlap

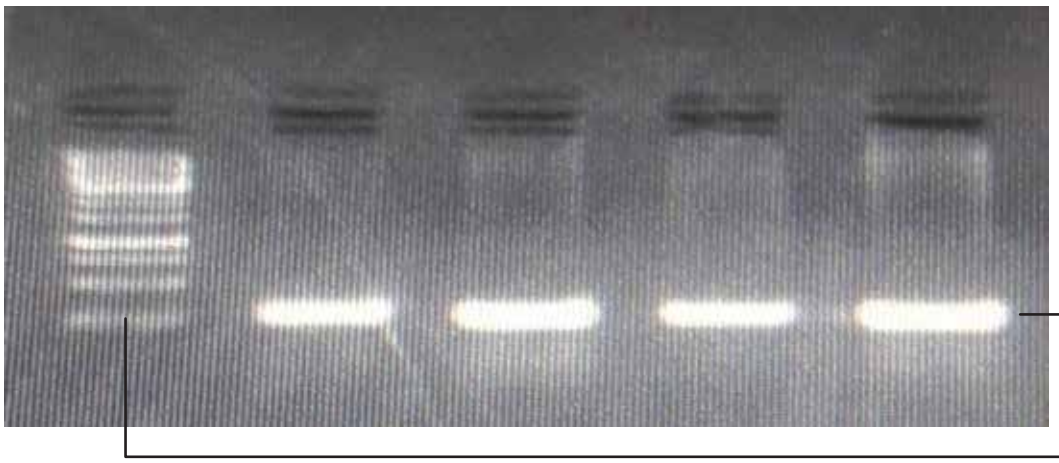
Serum vitamin D_3 status in different genotypes of FokI

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 D_3 status among subjects with different genotypes of FokI (n=47)

Genotypes	Serum 25(OH)D (ng/ml) mean±SEM	p-value
FF (n=42)	17.29±0.69	
Ff (n=3)	18.67±2.31	0.88
ff(n=2)	17.60±4.60	

Statistical analysis was done by ANOVA.
n=number of subjects



Marker	:	250 bp DNA ladder
Lane 1, 3	:	RFLP product (272bp)
Lane 2, 4	:	PCR product (272 bp)
bp	:	Base pair

Figure-1: Restriction endonuclease digestion for FokI

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 Bangladesh[22,23] and other countries[24] as well as in ACO patients[13]. Indoor staying from 10 AM to 2PM [25] and darker skin type[25,26] of our study subjects might be obstacle for conversion of 7-dehydrocholesterol to pre vitamin D_3 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)D[24], 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 D_3 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 D_3 status was different from us [27]. On the other hand, significant ($p<0.001$) association of frequency of FokI genotype with D_3 status was reported in Pakistani healthy women[28]. No significant difference was observed in the mean serum D_3 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 D_3 deficiency and FokI VDR SNP was not associated with D_3 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, Manirujjaman M, et al. Vitamin D and Parathyroid Hormone Status in Female

Garment Workers: A Case-Control Study in Bangladesh. Biomed Res Int. 2017;2017: 4105375. doi: 10.1155/2017/4105375.

2. Ponsonby AL, Lucas RM, van der Mei IA. UVR, vitamin D and three autoimmune diseases--multiple sclerosis, type 1 diabetes, rheumatoid arthritis. Photochem Photobiol. 2005 Nov-Dec;81(6):1267-75. doi: 10.1562/ 2005-02-15-IR-441
3. Holick MF. Vitamin D deficiency. N Engl J Med. 2007 Jul 19;357(3):266-81. doi: 10.1056/NEJMra070553.
4. Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. Am J Clin Nutr. 2008 Apr; 87(4):1080S-6S. doi:10.1093/ajcn/87.4. 1080S.
5. Pilz S, Tomaschitz A, Ritz E, Pieber TR. Vitamin D status and arterial hypertension: a systematic review. Nat Rev Cardiol. 2009 Oct;6(10):621-30. doi: 10.1038/nrcardio. 2009.135.
6. Yamshchikov AV, Desai NS, Blumberg HM, Ziegler TR, Tangpricha V. Vitamin D for treatment and prevention of infectious diseases: a systematic review of randomized controlled trials. Endocr Pract. 2009 Jul-Aug;15(5):438-49. doi: 10.4158/EP09101. ORR.
7. Black PN, Scragg R. Relationship between serum 25-hydroxyvitamin d and pulmonary function in the third national health and nutrition examination survey. Chest. 2005 Dec;128(6):3792-8. doi: 10.1378/chest.128. 6.3792
8. Heidari B, Javadian Y, Monadi M, Dankob Y, Firouzjahi A. Vitamin D status and distribution in patients with chronic obstructive pulmonary disease versus healthy controls. Caspian J Intern Med. 2015 Spring;6(2):93-7.
9. Janssens W, Bouillon R, Claes B, Carremans C, Lehouck A, Buysschaert I,et al.Vitamin D deficiency is highly prevalent in COPD and correlates with variants in the vitamin D-binding gene. Thorax. 2010 Mar;65(3): 215-20. doi:10.1136/thx.2009.120659
10. Ginde AA, Mansbach JM, Camargo CA Jr. Vitamin D, respiratory infections, and asthma. CurrAllergy Asthma Rep. 2009 Jan;9(1):81-7. doi: 10.1007/s11882-009-0012-7.
11. Brehm JM. Vitamin D and asthma-life after VIDA? Curr Allergy Asthma Rep. 2014 Sep;14(9):461. doi: 10.1007/s 11882-014-0461-5.
12. Jolliffe DA, Greenberg L, Hooper RL, Griffiths CJ, Camargo CA Jr, Kerley CP, et al. Vitamin D supplementation to prevent asthma exacerbations: a systematic review and meta-analysis of individual participant data. Lancet Respir Med. 2017 Nov;5(11):881-890. doi: 10.1016/S2213-2600(17)30306-5.
13. Odler B, Ivancsy I, Somogyi V, Benke K, Tam6si L, G6lffy G, et al.Vitamin D deficiency is associated with impaired disease control in asthma-COPD overlap syndrome patients. Int J Chron Obstruct Pulmon Dis. 2015 Sep 24;10:2017-25. doi: 10.2147/COPD.S91654.
14. Darraji SZL, Azzawie HFL, Kharsani AML. Associations between TaqI Polymorphisms in Vitamin D Receptor Gene and Type 2 Diabetes Mellitus in Obese Iraqi Population. J Diabetes Metab.2017. 8(4): 1- 4. doi:10.4172/ 2155-6156.1000734
15. Dabirnia R, Mahmazi S, Taromchi A, Nikzad M, Saburi E. The relationship between vitamin D receptor (VDR) polymorphism and the occurrence of osteoporosis in menopausal Iranian women. Clin Cases Miner Bone Metab. 2016 Sep-Dec;13(3):190-194. doi: 10.11138/ccmbm/2016.13.3.190.
16. Ilhan M, Toptas-Hekimoglu B, Yaylim I, Turgut S, Turan S, Karaman O, et al. Investigation of the vitamin D receptor polymorphisms in acromegaly patients. Biomed Res Int. 2015;2015:625981. doi: 10.1155/2015/625981.
17. Rashedi J, Asgharzadeh M, Moaddab SR, Sahebi L, Khalili M, Mazani M, et al. Vitamin d receptor gene polymorphism and vitamin d plasma concentration: correlation with susceptibility to tuberculosis. Adv Pharm Bull. 2014 Dec;4(Suppl 2):607-11. doi: 10.5681/apb.2014.089.
18. Hopkinson NS, Li KW, Kehoe A, Humphries SE, Roughton M, Moxham J, et al. Vitamin D receptor genotypes influence quadriceps strength in chronic obstructive pulmonary disease. Am J Clin Nutr. 2008 Feb;87(2):385-90. doi: 10.1093/ ajcn/87.2.

385.

19. Saadi A, Gao G, Li H, Wei C, Gong Y, Liu Q. Association study between vitamin D receptor gene polymorphisms and asthma in the Chinese Han population: a case-control study. BMC Med Genet. 2009 Jul 21; 10:71. doi: 10.1186/1471-2350-10-71.
20. Zhao DD, Yu DD, Ren QQ, Dong B, Zhao F, Sun YH. Association of vitamin D receptor gene polymorphisms with susceptibility to childhood asthma: A meta-analysis. Pediatr Pulmonol. 2017 Apr;52(4):423-429. doi: 10.1002/ppul.23548.
21. Papadopoulou A, Kouis P, Middleton N, Kolokotroni O, Karpathios T, Nicolaidou P, et al. Association of vitamin D receptor gene polymorphisms and vitamin D levels with asthma and atopy in Cypriot adolescents: a case-control study. Multidiscip Respir Med. 2015 Sep 4;10(1):26. doi: 10.1186/s 40248-015-0025-0.
22. Zaman S, Hawlader M D H, Biswas A, Hasan M, Jahan M, Ahsan G U.High Prevalence of Vitamin D Deficiency among Bangladeshi Children: An Emerging Public Health Problem. Health. 2017;9(12): 1680-1688. 10.4236/health.2017.912123
23. Hossain H A,Islam Q A, Khandaker M A K, Ahasan H N. Study of Serum Vitamin D Level in Different Socio-Demographic Population- A Pilot Study. J Medicine. 2018;19: 22-29
24. Kassi EN, Stavropoulos S, Kokkoris P, Galanos A, Moutsatsou P, Dimas C, et al. Smoking is a significant determinant of low serum vitamin D in young and middle-aged healthy males. Hormones (Athens). 2015 Apr-Jun;1 4(2): 245-50. doi: 10.14310/horm.2002.1521.
25. Engelsen O. The relationship between ultraviolet radiation exposure and vitamin D status. Nutrients. 2010 May;2(5):482-95. doi: 10.3390/nu2050482.
26. Webb AR, Engelsen O. Calculated ultraviolet exposure levels for a healthy vitamin D status. Photochem Photobiol. 2006 Nov-Dec; 82(6):1697-703. doi: 10.1562/2005- 09-01-RA-670.
27. Bhanushali AA, Lajpal N, Kulkarni SS, Chavan SS, Bagadi SS, Das BR. Frequency of fokI and taqI polymorphism of vitamin D receptor gene in Indian population and its association with 25-hydroxyvitamin D levels. Indian J Hum Genet. 2009 Sep;15(3):108-13. doi: 10.4103/ 0971-6866.60186.
28. Younus A, Faiz M, Yasmeen A. Association of vitamin D receptor FokI and TaqI gene polymorphisms in Pakistani women with 25(OH)D levels. Journal of Food and Nutrition Research.2017; 5(7):475-480.doi:10.12691/ jfnr-5-7-4.