Published in final edited form as:
Cancer. 2014 May 1; 120(9): 1387–1393. doi:10.1002/cncr.28565.

Vitamin D receptor Fok1 gene polymorphisms may be associated with CRC among African American and Hispanic participants

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Abstract

Background—Vitamin D plays a role in cancer tumorogenesis and acts through the vitamin D receptor (VDR). Although African Americans have the lowest levels of vitamin D serum levels, supplementation has not yielded significant improvement in cancer. Gene polymorphisms in VDR may play a role. There is dearth of information on VDR gene polymorphisms and colorectal cancer (CRC) among underrepresented ethnic groups. This study examines whether VDR SNPs, single-nucleotide-gene-polymorphisms, are associated with CRC in predominately African American and Hispanic study participants.

Methods—Blood was collected from 378 participants, with 78 colorectal-cancer patients (Cases), 230 non-cancer subjects with no polyps (Controls w/o polyps), and 70 non-cancer subjects with polyps (Controls w/polyp). The four polymorphic SNPs in VDR (FokI, BsmI, TaqI, ApaI) were assessed using the PCR-RFLP method.

Results—There was significant association of the VDR-Fok1 FF genotype with CRC Cases (OR=2.9; P=0.036) when compared with Controls w/o polyps. The most common VDR-Fok1 genotype in the overall study population was the FF genotype (46%). However, upon breakdown by ethnicity, the FF was the most common in African American participants (61%), and the Ff was most common in Hispanic/Latino participants (49%). When the association was assessed in a multivariate model, there was no significant association with any VDR polymorphism and CRC Cases (P>0.05). The other three polymorphic variants of VDR (BsmI, TaqI and ApaI) were not associated with CRC.

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Disclosure: The authors have no significant financial conflicts to disclose.

Conclusions—This study suggests that genetic variation of the VDR-FokI SNPs may influence CRC risk, particularly in African American cohorts.

Keywords

Vitamin D receptor; genetic polymorphism; African American; Hispanic; Colorectal Cancer

INTRODUCTION

Colorectal cancer (CRC) is the third highest leading cause of cancer-related mortality in the United States[1]. Among all ethnic groups, African Americans have the highest mortality rates and have a higher burden of cancer health disparities[1]. While tumor biology and socio-demographic factors such as access to screening and care may play a role, more recent studies have focused on modifiable factors which may help attenuate disparities in morality outcomes. Specifically, there have been several studies, randomized clinical trials, and reviews which have presented substantial evidence that vitamin D levels may play a significant role in CRC risk and mortality[2–6]. Since African American, as well as Hispanic/Latino, groups have significantly lower levels of serum vitamin D[7–9], this may be a health disparity factor whereby vitamin D contributes to higher incidence and mortality among these groups[10].

To date, vitamin D supplementation studies, often paired with calcium supplementation, have yielded some promising results in reducing biomarkers associated with colonic adenoma recurrence[3, 11, 12]. However, the effects of vitamin D supplementation on reducing relative risk of CRC in carefully controlled clinical trials have not shown significant results leaving the clinical applicability of vitamin D supplementation inconclusive[13, 14]. Understanding variations in the vitamin D signaling pathway, particularly through the vitamin D receptor (VDR) may provide insights into other factors which may contribute to the efficacy of vitamin D[15]. Defective alterations in VDR expression and activity could lead to abnormal vitamin D uptake, metabolism, and serum levels of biologically active vitamin D[15]. The VDR gene has been shown to have multiple gene polymorphisms[2] with four important single nucleotide polymorphisms (SNPs) as follows: VDR-FokI (rs2228570; C>T), VDR-BsmI (rs1544410; A>G), VDR-TaqI (rs731236; C>T) and VDR-ApaI (rs7975232; A>C). Recent functional studies on VDR SNPs have identified that the VDR-Fok1 polymorphism is in a coding region of the VDR gene and leads to a shorter VDR protein by altering the transcription initiation site[16, 17].

Comprehensive reviews of the literature have resulted in an inconclusive association of VDR SNPs with CRC[2]. Some studies show an inverse correlation[18, 19] while others show direct correlation[20, 21]. Meta-analysis studies have suggested significant association of VDR polymorphisms with CRC and other cancers across multiple cohorts[14, 22]. However, these analyses were primarily on Caucasian or Asian cohorts, and did not include significant numbers of African American and Hispanic/Latino participants[22]. Hence, the aim of the present study is to investigate the association between VDR gene polymorphisms and CRC among African American and Hispanic participants. To the knowledge of the authors, this will be among the first studies focusing on VDR polymorphisms and CRC in this

underrepresented participant cohort. Identifying whether VDR polymorphisms exert a role on CRC may inform future vitamin D supplementation studies to optimize treatment for patients with hypovitaminosis D. The implications of optimized vitamin D treatment among populations with low vitamin D have the potential to substantially impact ethnic disparity in CRC mortality.

METHODS

Ethics Statement

The study was approved by the Charles R. Drew University of Science and Medicine Institutional Review Board. Informed consent was obtained from all participants.

Study Population

This is a retrospective cohort hospital-based study. Subjects were selected from our current colorectal study database from the Division of Cancer Research and Training, at Charles R. Drew University of Medicine and Science. The selection was as follows: a) participant had a biopsy confirmed neoplasm; b) participant had a colonoscopy and was confirmed to be free of polyps; c) participant had a colonoscopy with biopsy of the polyps confirmed to be non-cancer; d) participant had documented personal history, clinical history, and tumor pathology data; and e) participant had availability of high quality DNA extracted from blood sample. A total of 378 participants were included in the study, with 78 (20.6%) CRC cases (“Cases”), 230 (60.8%) controls without polyps (“Controls w/o polyps”), and 70 (18.6%) controls with polyps (“Controls w/ polyps”). Body mass index was calculated as kg/m^2.

VDR polymorphisms

DNA was extracted from buffy coat samples utilizing the Qiagen DNA extraction kit. Polymerase chain reaction-restriction fragment length polymorphism analysis (PCR-RFLP) was performed utilizing flanking primers[23, 24] to genotype the four VDR single-nucleotide-polymorphisms (SNPs). The VDR-FokI polymorphic site was amplified using the following primers; Forward -5’GATGCCAGCTGGCCCTGGCACTG3’ and Reverse – 5’ATGGAAACACCTTGCTTCTTCTCCCTC 3’. The VDR-ApaI and VDR-TaqI polymorphisms were detected using one primer in intron 8 (Forward: 5’-AGAGCATGGACAGGGAGCAAG- 3’) and the other in exon 9 (Reverse: 5’-GCAACTCCTCATGGCTGAGGTCTCA- 3’), resulting in a 745 bp fragment spanning the ApaI and TaqI site. The VDR-BsmI polymorphism was detected with one primer originating in exon 7 (Forward: 5’-CAACCAAGACTACAAGTACCGCGTCAGTGA-3’) and a second in intron 8 (Reverse: 5’-AACCAGCGGAAGAGGTCAAGGG-3’), amplifying an 823 base pair (bp) fragment spanning the BsmI site. PCR was performed by denaturation at 94○C for 5 minutes, followed by 40 cycles of PCR at 94○C (30 seconds), 60○C-63.5○C (30 seconds), and 72○C (30 seconds). The annealing temperatures were 59.5○C for FokI, 61○C for ApaI/ TaqI, and 63.5○C for BsmI.

The amplified PCR products were digested with the FokI, ApaI, TaqI, and BsmI enzymes as per the manufacturer’s specifications (New England Biolabs, Beverly, MA, USA). The enzyme recognition sites were identified by ethidium bromide staining of fragments

separated in a 2% agarose gel. The PCR products of the VDR-FokI polymorphism resulted in a 273bp fragment, which upon digestion could provide a 198bp and 75bp product in the presence of restriction site. The PCR products containing the polymorphic cut sites digested with TaqI enzyme resulted in fragments of 496bp and 249bp, and the PCR products digested with ApaI enzyme resulted in fragments of 531bp and 214bp. The BsmI cleaved PCR product produced either the single resultant fragment at 823bp, or the two resultant fragments at 648bp and 175bp. The polymorphisms are presented as FF, Ff and ff for the VDR-FokI polymorphism, AA, Aa, aa for the VDR-ApaI polymorphism, TT, Tt and tt for the VDR-TaqI polymorphism, BB, Bb, bb for the VDR-BsmI polymorphism.

Tumor clinicopathological features (TNM) are described according to the American Joint Committee on Cancer (AJCC) definitions. Duke Stage was defined as A (Stage I), B (Stage II), C (Stage III), and D (Stage IV)[25].

Statistical Analysis

The data for this study was analyzed with SPSS Software (IBM, New York) versions 11.5–20.0. The Pearson Chi-square test was used to determine statistically significant differences in the frequency distribution of VDR genotypes between Cases and Controls. If the Table had cells with a frequency less than 5, Fisher's exact test was utilized. The 2-sided exact p-value <0.05 was considered statistically significant. Associations between each VDR polymorphism and CRC were estimated using the Logistic Regression test at the 95% confidence interval. For each polymorphic site, the referent genotype is indicated for the analysis within each Table, since reference genotypes varied among populations and between studies in published literature[22].

RESULTS

We had a total of 378 participants in the study, with 78 CRC cases, 230 Controls w/o polyps, and 70 Controls w/polyps.

Descriptive Characteristics

The descriptive characteristics of the study participants in the study are shown in Table 1. A total of 149 (39.4%) participants self-identified as African American, 212 (56.1%) as Hispanic/Latino, 9 (2.4%) as Caucasian, and 8 (2.1%) as Asian. The gender distribution of the participants included 159 (42%) male, and 19 (58%) female. The overall mean age of participants was 55 years-old. The mean age of diagnosis with cancer was 55 years old and the mean age for non-cancer participants receiving colonoscopy was 55–56 years old. The mean BMI for participants was 28.1kg/m^2.

Ethnic Distribution of VDR Polymorphisms

The distribution of the VDR polymorphisms (Fok1, Bsm1, Taq1, Apa1) are shown based on ethnicity in Table 2. Overall, the most common genotype for each of the polymorphisms was FF for VDR-Fok1 (46%), bb for VDR-Bsm1 (48%), Aa for VDR-Apa1 (52%), and TT for VDR-Taq1 (62%). In the VDR-Fok1, FF was most common for African American participants (61%) and Asians (75%); however, Ff was most common among Hispanic/

Latinos (50%) and Caucasians (67%). In the VDR-Bsm1 polymorphism distribution, Bb was most prevalent among African Americans (49%) while bb was most common in all others. Similarly, the VDR-Apa1 polymorphism AA was more common in African Americans (49%), while Aa was the most frequent for the other ethnicities. The VDR-Taq1 TT genotype was most common for all the ethnicities. The P-value for the distribution between African American and Hispanic/Latino participants was assessed separately due to the lower numbers of Asian and Caucasian participants. There were statistically significant differences between the distribution of the VDR-Fok1 (P=0.006) and VDR-Apa1 polymorphisms (P=0.009).

Univariate Analysis on VDR polymorphisms and CRC

Analysis on the relationship between VDR polymorphisms and CRC was performed first by univariate analysis. The results are shown in Table 3. The FF genotype of the VDR-Fok1 was significantly associated with CRC cases (OR=2.9; 95%CI=1.1–8.0; P=0.036). None of the other VDR-polymorphisms were significantly associated with CRC cases.

Multivariate Analysis on VDR polymorphisms and CRC

Analysis adjusting for ethnicity, gender, age, BMI, and cancer diagnosis was performed in the multivariate model shown in Table 4. There were no statistically significant associations of VDR-polymorphisms in the adjusted model with CRC cases.

VDR Haplotypes and CRC

Haplotype analysis of the polymorphic variants resulted in 16 different haplotypes as shown in Table 5. There was no significant associations of any of the haplotypes with CRC among African American participants (P>0.05). However, there was a statistically significant trend association of the fbat haplotype genotype with CRC among Hispanic/Latino participants (OR=1.1; 95%CI=1.0–1.2; P=0.048).

VDR Polymorphisms and CRC Clinicopathology

None of the four VDR-polymorphism groups (Fok1,Bsm1,Apa1,Taq1) were associated with the Duke stage, tumor size (T), or lymph node involvement (N) (data not shown). However, there was a statistically significant association (P=0.011) of the VDR-Fok1 genotype ff polymorphism with metastasis status (M) (data not shown).

DISCUSSION

To the knowledge of the authors, this is one of the first studies to assess vitamin D receptor gene polymorphisms in association with colorectal cancer among African American and Hispanic/Latino participants. With a study size of 378 participants, our study provides much needed information to aid in bridging the gap in scientific knowledge on the role of VDR gene polymorphisms and CRC among underrepresented cohorts. Mortality and outcome from CRC is a significant source of cancer health disparities, with highest incidence and mortality among African Americans[1]. Vitamin D has been identified as an important factor, potentially modifiable, which significantly contributes to both CRC risk[3, 11, 12] and

outcome[6]. However, vitamin D supplementation studies have not yielded conclusive results according to an extensive review by Buttigliero and colleagues[14].

The present study identified data relevant to the role of VDR and CRC among underrepresented populations. Foremost, the distribution of the VDR polymorphism alleles was different between African American and Hispanic study participants. There was a higher frequency of the VDR-Fok1 FF genotype among African American individuals compared with Hispanic participants. The FF genotype was a statistically significant polymorphism associated with colorectal cancer in this study. These data are consistent with a previous study conducted by the present authors among a similar population of African American and Hispanic women with breast cancer[26]. An association was identified with the Fok1 polymorphism and breast cancer among African American women (OR=1.9, p=0.07). The Fok1 FF genotype was furthermore associated with reduced disease free survival among breast cancer patients[26]. Cox regression with multivariate analysis demonstrated the independently predictive value of VDR-FokI on disease free survival from breast cancer. The association of the Fok1 polymorphism with clinical outcome suggests that there may be a key biological role that the Fok1 polymorphism affects in the VDR. Alterations in VDR and VDR activity due to the Fok1 polymorphisms may influence potential response to vitamin D and play a role in the efficacy of supplementation studies. Within the context of the present CRC and VDR study, these potential associations must be interpreted cautiously since the significance of the Fok1 polymorphism in relation to CRC was lost in the multivariate analysis. This may have been due to the limited number of cases in the study and sets a precedent to merit investigation in a larger cohort of underrepresented African American and Hispanic/Latino participants.

The implications of VDR-Fok1 polymorphisms on colorectal cancer, however, are supported in other studies. A case-control study conducted by Ochs-Balcom[20] and colleagues observed an increased risk of CRC associated with the Fok1 FF genotype (OR=1.87, 95% CI=1.03–3.38). The OR in our study is significantly higher (2.9vs.1.87). Sweeney[21] et al also identified that the two haplotypes that included the Fok1 F allele were associated with increased risk. Park[19] et al conducted a population-based study of VDR genotypes and CRC in South Korea on 190 CRC cases compared to 318 healthy controls with no history of CRC. CRC cancer risk was significantly lower among those with the FokI ff genotype (compared to FF) (OR=0.35, 95% CI=0.19–0.65). Similarly, our data showed significant association of Fok1 FF genotype with colorectal cancer.

These data together suggest that the VDR-Fok1 polymorphism may be an important factor to consider in assessing both CRC risk and outcome, particularly among African American participants who have higher frequency of the FF genotype associated with risk/poor outcome. VDR polymorphisms were assessed in the context of stage and clinicopathology. A significant association with metastatic status was identified (data not shown). However, these data merit further investigation in a larger cohort of African American participants. Furthermore, functional studies are necessary in order to confirm the role of the Fok1 genotype in vitamin D receptor mediated signaling in CRC cell models, although it has been confirmed in breast cancer[16].

The other VDR polymorphisms-Bsm1, Apa1, Taq1-were not significantly associated with any factors in the present study. An association of the Apa1 polymorphism among Hispanic/Latina women and breast cancer observed in our breast study[26] was not similarly identified in the CRC study. This may be due to the smaller number of Cases in the CRC study. Interestingly, a recent meta-analysis by Bai and colleagues had identified a significant association of only the Bsm1 polymorphism (compared with Fok1, Apa1, Taq1, and others) with CRC risk[27]. However, the study included all the diverse participants (African American, Hispanic American, European American, Asian American, etc.) under a single category as "American" decent which may have affected the results and conclusions drawn from the analysis. In fact, the lack of significant numbers of ethnically diverse participants (<1–5%) in several previously reported studies[3, 20, 28–32] and meta-analysis[33] may contribute to the conflicting reports identified associating VDR polymorphisms with CRC risk.

Some polymorphisms may be present in higher frequency among certain ethnic groups, and may therefore exert a more important role, paired with hypovitaminosis D, to alter risk. The study by Murtaugh[18] and colleagues was among the few studies which included >5% African American and Hispanic American participants and significantly identified an association of the VDR-Fok1 polymorphisms (FF genotype) with CRC. A recent large meta-analysis/review of VDR polymorphisms and cancer risk conducted by Raimondi and colleagues[22] included analysis by ethnicity, and identified no significant association of CRC risk with the VDR-Fok1 polymorphism. However, the review included no studies assessing VDR polymorphisms and CRC in African American or Hispanic/Latino populations – the reported analysis was only on Caucasian and Asian participants[22]. Therefore, the present study is among the first to report VDR polymorphisms in African American and Hispanic/Latino cohorts in the context of CRC and can help bridge the understanding of trends/association in these cohorts.

In conclusion, studies have identified that vitamin D levels play a role in CRC risk and progression; however, utilizing vitamin D supplementation to overcome vitamin D insufficiency has not resulted in unequivocal improvement. Hence, examination of polymorphisms potentially impacting VDR protein function may be central in identifying confounding factors that play a role in vitamin D activity. Ultimately, these polymorphisms may be used as biomarkers for informing vitamin D supplementation studies, with the potential to impact patient risk and outcome from CRC. The present study is among the first to report VDR polymorphisms in African American and Hispanic/Latino participants in the context of CRC. The findings on these VDR variants, particularly in VDR-Fok1, suggest the potential need to screen for VDR polymorphisms in conjunction with screening vitamin D serum levels. As a potentially modifiable risk factor, vitamin D and the VDR mediated axis may be a point of significant interventional opportunity to effectively reduce CRC risk, with the overarching goal to reduce cancer health disparities. Hence, additional studies with a larger sample size are warranted to better understand the vitamin D axis and risk for CRC.

Acknowledgments

The authors would like to thank all of the participants in our study.

Funding: Support from NIH - Grant numbers: NCI U56CA101599-01; CA15083-25S3; U54CA14393; NIMHD U54MD007598; and NCATS CTSI UL1TR000124 to J.V.V. NIH/NIMHD CRECD 5MD007610 to Y Wu.

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Table 1

Characteristics of Colon Study Participants

	Total	Colon Cancer	Non-Cancer	Non-cancer w/polyps
N (%)	**378 (100)**	**78 (20.6)**	**230 (60.8)**	**70 (18.6)**
Ethnicity				
African American	149 (39.4)	41 (52.6)	79 (34.3)	29 (41.4)
Hispanic/Latino	212 (56.1)	27 (34.6)	144 (62.6)	41 (58.6)
Caucasian	9 (2.4)	5 (6.4)	4 (1.7)	0
Asian	8 (2.1)	5 (6.4)	3 (1.3)	0
Gender				
Male	159 (42.1)	43 (55.1)	85 (37.0)	31 (44.3)
Female	219 (57.9)	35 (44.9)	145 (63.0)	39 (55.7)
Age at Dx†				
Mean±SD	55.2±9.9	55.1±10.2	54.9±9.8	56.2±10.2
Body Mass Index				
Mean±SD	28.1±8.4	26.3±8.3	29.1±7.9	27.3±9.8

†Age of Dx refers to Age of diagnosis for CRC patients, and age at consent for non-cancer patients.

SD indicates Standard Deviation.

Table 2

Distribution of Fok1, Bsm1, Apa1, and Taq1 Genotypes

	Total	African American	Hispanic-Latino	Caucasian	Asian	*P-Value
	N (%)	N (%)	N (%)	N (%)	N (%)	
Fok1	N = 375	N=150	N=208	N=9	N=8	
FF	172 (45.9)	92 (61.3)	71 (34.1)	3 (33.3)	6 (75.0)	**0.02**
Ff	160 (42.7)	49 (32.7)	103 (49.5)	6 (66.7)	2 (25.0)	
ff	43 (11.5)	9 (6.0)	34 (16.3)	0	0	
			^P=0.006			
Bsm1	N=378	N=151	N=210	N=9	N=8	
BB	37 (9.8)	18 (11.9)	15 (7.1)	4 (44.4)	0	**0.02**
Bb	159 (42.1)	74 (49.0)	82 (39.0)	1 (11.1)	2 (25.0)	
bb	182 (48.1)	59 (39.1)	113 (53.8)	4 (44.4)	6 (75.0)	
			^P=0.09			
Apa1	N=370	N=147	N=207	N=8	N=8	
AA	133 (35.9)	72 (49.0)	57 (27.5)	2 (25.0)	2 (25.0)	**<0.001**
Aa	192 (51.9)	61 (41.5)	123 (59.4)	4 (50.0)	4 (50.0)	
aa	45 (12.2)	14 (9.5)	27 (13.0)	2 (25.0)	2 (25.0)	
			^P=0.009			
Taq1	N=371	N=147	N=207	N=9	N=8	
TT	229 (61.7)	84 (57.1)	132 (63.8)	6 (66.7)	7 (87.5)	**0.033**
Tt	111 (29.9)	47 (32.0)	61 (29.5)	2 (22.2)	1 (12.5)	
tt	31 (8.4)	16 (10.9)	14 (6.8)	1 (11.1)	0	
			^P=0.908			

*P-Value: Comparison between four ethnic groups.

^P-Value: African American vs. Hispanic-Latino.

P-value<0.05 is significant (in Bold).

Table 3

Odds Ratios Assessing the Association between VDR gene polymorphisms and Colorectal Cancer (Univariate Analysis)

	(Colon Cancer vs. Normal)			(Non-Cancer w/polyps vs. Normal)		
	N	**OR (95% CI)**	**P-value**	**N**	**OR (95% CI)**	**P-value**
Fok1						
FF	44/99	**2.9 (1.1–8.0)**	**0.036**	29/99	1.9 (0.7–5.4)	0.209
Ff	29/99	1.9 (0.7–5.4)	0.209	32/99	2.1 (0.8–5.9)	0.146
ff	5/33	1	5/3	1		
Bsm1						
BB	9/23	1.2 (0.5–2.7)	0.729	5/23	0.68 (0.2–1.9)	0.473
Bb	32/98	0.97 (0.6–1.7)	0.915	29/98	0.93 (0.5–1.6)	0.8
bb	37/110	1		35/110	1	
Apa1						
AA	28/78	0.93 (0.4–2.2)	0.873	41/140	2.1 (0.6–7.5)	0.233
Aa	37/123	0.78 (0.3–1.8)	0.555	24/63	2.8 (0.8–10.1)	0.12
aa	10/26	1		3/22	1	
Taq1						
TT	44/140	1.3 (0.5–3.3)	0.873	27/78	1.0 (0.4–2.4)	1
Tt	24/63	1.4 (0.5–3.8)	0.52	32/123	0.8 (0.3–1.8)	0.51
tt	6/22	1		9/26	1	

*P-value <0.05 is significant (in Bold). OR indicates odds ratio; CI, confidence interval.

Table 4

Odds Ratios for the association between VDR gene polymorphisms and colon cancer risk (*Multivariate Analysis)

Polymorphisms	OR (95% CI)	P-Value
Fok1		
FF	2.1 (0.2–18.2)	0.51
Ff	2.0 (0.2–17.9)	0.53
ff	1.0 (Ref)	
Bsm1		
BB	1.3 (0.3–5.6)	0.76
Bb	0.8 (0.3–2.7)	0.75
bb	1.0 (Ref)	
Apa1		
AA	0.6 (0.1–2.6)	0.51
Aa	0.6 (0.1–2.8)	0.47
aa	1.0 (Ref)	
Taq1		
TT	1.5 (0.2–13.8)	0.69
Tt	1.8 (0.2–15.6)	0.59
tt	1.0 (Ref)	

OR indicates odds ratio; CI, confidence interval; Ref, reference category. P-value < 0.05 is significant.

*Adjusted for ethnicity, gender, age, BMI and non-cancer status with polyps.

Table 5

Association of VDR haplotypes and Colon Cancer in African American and Hispanic/Latino Study Participants

	African American			Hispanic/Latino		
VDR Haplotypes	**OR**	**(95% CI)**	**P-value**	**OR**	**95% CI**	**P-value**
FBTA	1.00 (Ref)	----	----	1.00(Ref)	----	----
FbAT	1.30	(0.6–2.9)	0.539	1.60	(0.6–4.7)	0.355
FbAt	1.20	(0.6–2.5)	0.528	1.20	(0.4–3.7)	0.798
fbAt	----	----	----	0.97	(0.5–2.1)	0.931
fbAT	1.10	(0.8–1.6)	0.566	0.96	(0.7–1.4)	0.836
FbaT	1.10	(0.9–1.3)	0.560	1.20	(0.9–1.5)	0.163
FBAt	1.00	(0.8–1.2)	0.960	0.87	(0.6–1.2)	0.434
fBAT	0.96	(0.7–1.4)	0.839	1.20	(0.9–1.5)	0.222
fBAt	0.94	(0.8–1.2)	0.589	----	----	----
fbaT	0.96	(0.8–1.1)	0.576	0.96	(0.8–1.1)	0.494
FBaT	----	----	----	----	----	----
Fbat	0.93	(0.8–1.1)	0.339	0.99	(0.8–1.2)	0.931
fBat	----	----	----	----	----	----
fBaT	----	----	----	----	----	----
FBat	----	----	----	----	----	----
fbat	0.98	(0.9–1.1)	0.738	**1.1**	**(1.0–1.2)**	**0.048**

OR indicates odds ratio; CI, confidence interval; Ref, reference category. P-value<0.05 is significant(In Bold).