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RESEARCH ARTICLE

ASSOCIATION OF VITAMIN D RECEPTOR BSMI POLYMORPHISM WITH SUSCEPTIBILITY TOTUBERCULOSIS AND TYPES OF TUBERCULOSIS INFECTION AMONG SUDANESE PATIENTS

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ABSTRACT

Background: A case-control study was conducted to study vitamin D receptor VDR-BsmI polymorphism with tuberculosis (TB) susceptibility in the different types of tuberculosis infection among Sudanese patients. **Methods:** One hundred and twenty tuberculosis patients with 46 apparently healthy controls were included for genotyping of the VDR-BsmI polymorphism using Polymerase chain reaction and restriction fragment length polymorphism (PCR –RFLP). **Results:** This study found that the BsmI polymorphism in VDR gene is significantly associated with tuberculosis infection (P=0.00) and the heterozygous allele Bb is associated with susceptibility to tuberculosis infection with odd ratio 4.681. The pulmonary tuberculosis was the most predominant in the study population and it was found to be associated with the VDR-BsmI heterozygous Bb allele odds ratio (OR) 1.319 while the bb allele was associated with the Extra-pulmonary tuberculosis infections. **Conclusion:** The b allele is associated with susceptibility to TB infections among Sudanese patients especially to the extra pulmonary TB.

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INTRODUCTION

Tuberculosis (TB) is a chronic infectious disease occurs when the M. tuberculosis is inhaled causing pulmonary tuberculosis and can localize in alternate sites leading to extra-pulmonary tuberculosis (EPTB) (Tandon, 1978). Extra-pulmonary TB accounts for approximately 10% of tuberculosis infections (Verettas *et al*., 2003) and Spinal TB accounts for about 2% of cases of TB (Snider, 1997). In the year 2015 there was new incident 10.4 million cases of TB with 1.4 million TB deaths worldwide (Who global tuberculosis report, 2016). Several studies showed genetic variants and regions of the genome associated with tuberculosis risk (Oki *et al*., 2011). Vitamin D receptor (VDR) interaction with the 1, 25-dihydroxyvitamin D3 (also known as Calcitriol) is able to activate monocytes, stimulate cellmediated immunity and suppress lymphocyte proliferation (Tachi *et al*., 2003). VDR gene mutation may affect the immunity activity and the subsequent mediated effect of VDR, it has been considered as a risk factor in TB development process. BsmI is one of the most frequently studied VDR polymorphisms which is located in intron 8 binding to the 3'UTR, and genotyped as BB, Bb, or bb. The VDR gene is located on chromosome 12-q-14 (Liza Bornman *et al*., 2004). The differentiation and growth of various immune cells is regulated by the Calcitrioland its derivatives have been shown to inhibit the functional differentiation of dendritic cells, cytotoxic T-cells, and helper T-cells (Lemire *et al*., 1995; Imazeki *et al*., 2006) also it suppresses the growth of Mycobacterium tuberculosis in mononuclear phagocytes9 and toll-like receptor activation of human macrophages has been shown to upregulate expression of VDR and vitamin D 1α-hydroxylase genes leading to induction of cathelicidin and killing of M. tuberculosis (Liu *et al*., 2006; Martineau *et al*., 2007).

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MATERIALS AND METHODS

Participants: This is a case control study was performed at the National Center for Neurological sciences(NCNS) during 2014-2016, Sudan on 120 Sudanese tuberculosis patients and 46 apparently healthy controls. The patients included were diagnosed with tuberculosis based on sign and symptoms, having evident lesions of TB by radiological and CT-scan and confirmed by positive PCR for *M.tuberculosis* from blood specimens (Mukhtar *et al*., 2013) (Pulmonary and extra-pulmonary according to the site of infection). Five mL of venous blood was collected into K3EDTA anticoagulant for DNA extraction for the genotyping. Genotype was performed by PCR – RFLP using the restriction enzyme BsmI.

Molecular analysis: The DNA was extracted using saturated salt methods then a reaction mix of 23µlfor the PCR was prepared using 4 µl of 5x PCR buffer (master mix ready to use from Solis BioDyne, ESTONIA), from the primers 0.75µl of each forward and reverse (10 pmol each), 3 µl the DNA, then the volume was completed to 23 µl bydistilled water. For the amplification DNA was denatured for 3 min at 94°C; 35 amplification cycles were performed with an automated thermal cycler (ESCO HEALTHCARE). Each cycle consisted of denaturation at 94°C for 30 sec, annealing of primers at 63°C for 30 sec extension at 72°C for 30 sec and primer final extension at 72°C for 8 min and finally holding temperature 4°C. The product band size for the BsmI is 823bp.

The PCR product of the 823bp band was digested with 1.0 unit of BsmI restriction enzyme (New England Biolabs, England)after digestion 5 µl of the mixture was loaded into 2% (agarose gel) containing ethidium bromide and visualized using gel documentation system UV transilluminator (Syngene). The sizes were determined using 100-bp ladder (SOLIS BIODYNE, ESTONIA). Primers used for the amplification of VDR, BsmI SNP. When the enzyme digests the product to 648 and 175bp then the genotype will be bb homozygous, if No cut so still 823 it is BB homozygous and if there was 823, 648 and 175 it is heterozygous Bb.

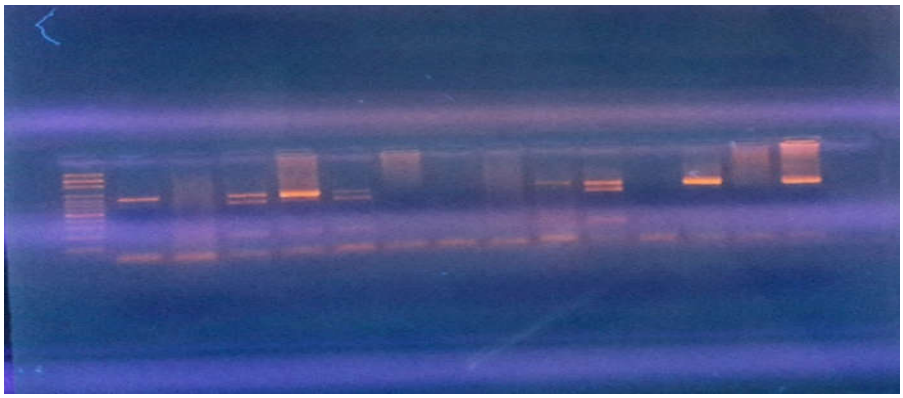
Statistical analysis: The association analyses were tested by the χ^2 test. Odds ratios and 95% confidence intervals were calculated from contingency tables. SNP(s) showing significant association ($p \leq 0.05$) in the tests. All statistical analyses were performed with SPSS 19.0 software (IBM SPSS Statistics 19).

Ethical considerations: Ethical clearance was taken from the Ethical Review Board of National Center for Neurological Sciences and verbal consent was obtained from each patient.

RESULTS

Demographic information: A total of 120 patients attended National center for Neurological Sciences (NCNS) and diagnosed with tuberculosis (Pulmonary and extra- pulmonary)

Forward	5′CAACCAAGACTACAAGTACCGCGTCAGTGA 3′
Reverse	5′-AACCAGCGGAAGAGGTCAAGGG-3



		Case				
		Patients (n:61)		Control (n:46)		
		Count(61)	Column N %	Count(46)	Column N %	P-value
BsmI	BB	22	36.1%	32	69.6%	.000
	bb	1	1.6%	2	4.3%	
	Bb	38	62.3%	12	26.1%	

Table 2. Frequency distribution of binary VDR BsmI Genotypes in patients and controls ((NCNS) 2014-2017, n: 120)

		Case					Odd Ratio
		Patients		Control			
		Count	Column N %	Count	Column N %	P-value	
BsmI BB	Present	22	36.1%	32	69.6%	.001	.247
	Absent	39	63.9%	14	30.4%		
BsmI bb	Present	1	1.6%	2	4.3%	.576	.367
	Absent	60	98.4%	44	95.7%		
BsmI Bb	Present	38	62.3%	12	26.1%	.000	4.681
	Absent	23	37.7%	34	73.9%		

Table 3. Frequency distribution binary VDR BsmI Genotypes in types of TB ((NCNS) 2014-2017, n: 120)

		TB					Odd Ratio
		Pulmonary Tuberculosis		Extra pulmonary TB			
		Count	Column N %	Count	Column N %	P-value	
BsmI BB	Present	13	35.1%	9	37.5%	1.00	.903
	Absent	24	64.9%	15	62.5%		
BsmI bb	Present	0	0.0%	1	4.2%	.393	.621
	Absent	37	100.0%	23	95.8%		
BsmI Bb	Present	24	64.9%	14	58.3%	.607	1.319
	Absent	13	35.1%	10	41.7%		

Table 4. In the gene BsmI the mutant genotype Bb are associated with TB infection Odd Ratio

		TB					Odd Ratio
		Extra-pulmonary TB		Pulmonary Tuberculosis			
		Count	Column N %	Count	Column N %	P-value	
BsmI BB	Present	9	37.5%	13	35.1%	1.00	1.1
	Absent	15	62.5%	24	64.9%		
BsmI bb	Present	1	4.2%	0	0.0%	.393	1.6
	Absent	23	95.8%	37	100.0%		
BsmI Bb	Present	14	58.3%	24	64.9%	.607	.76

and 46 apparently healthy blood donors were recruited as controls. 83 patients were (69.2%) males and 37 (30.8%) females and they were aging from 6 months to 90 years. In the studied group there were 66 (55%) with Pulmonary TB, 39(32.5%) with Pott's disease, 13(10.8%) with brain tuberculomata and 2(1.7%) with other types of TB (protenial and renal TB). The PCR for amplification of VDR polymorphism BsmI was made for all of the 120 patients but 59 gave no PCR product although all 120 were positive PCR for *M.tuberculosis*. No deviation was observed from Hardy-Weinberg equilibrium (HWE) in the genotypic distribution of the VDR-BsmI for the 61 patients.23(37.1%) out of 62 patients had the genotype BB, while 1(1.6%) had the mutant genotype bb and 38(61.3%) were heterozygous with the genotype Bb and 32(69.6%) out of 46 controls had the genotype BB, while 2(4.3%) had the mutant genotype bb and 12(26.1%) were heterozygous with the genotype Bb. The polymorphism BsmI is found to be associated with tuberculosis infection in this study with p value (0.000). The product band size is 823bp for vitamin D receptor VDR polymorphism BsmI and the pattern of digestion fragments using PCR-RFLP among the studied patients group ((NCNS) 2014-2017, n: 120). The genotype Bb is associated with the pulmonary Tuberculosis, OR= (1.319) Table (3) and the homozygous bb are associated with extra pulmonary tuberculosis OR 1.6.

DISCUSSION

In this study we found that the BsmI polymorphism in VDR gene is significantly associated with tuberculosis infection (P=0.00) when compared to the control and specifically the heterozygous allele Bb is associated with susceptibility to tuberculosis infection with odd ratio 4.681.these findings disagree with Bornman *et al* who genotyped the VDR single-nucleotide polymorphisms (SNPs) FokI, BsmI, ApaI, and TaqI and their analysis showed no statistically significant association between TB and VDR variants (Liza Bornman *et al*., 2004). Also disagree with Salimi *et al* (investigated the association between the VDR gene polymorphisms and pulmonary tuberculosis (PTB) in Iran (Salimi *et al*., 2015) who also found no association with the BsmI polymorphism, and both studies were done in different population from the Sudanese population .In our studythe pulmonary tuberculosis was the most predominant in the study population and it was found to be associated with the VDR-BsmI heterozygous Bb allele odds ratio (OR) 1.319 while the bb allele was associated with the Extra-pulmonary tuberculosis infections, that to some extend agrees with the findings of Singla *et al.* (2015) who analyzed the treatment outcome for the patients and reported that 'Bb' genotype is a risk in patients with unsuccessful treatment when compared to those with successful treatment. Since the vitamin D and its derivatives suppresses the growth of Mycobacterium tuberculosis in mononuclear phagocytes[(2)] and the findings of Joshi *et al.* (2014) that Vitamin D deficiency (VDD) is associated with an impaired mycobacterial immunity and susceptibility to tuberculosis and also the BB and Bb genotypes of BsmI were significantly associated in patients (P < 0.014; OR: 0.509; CI: 0.265-0.876) (P < 0.001; OR: 2.351; CI: 1.368-4.041), all these support with results.

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