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Association of *CYP2R1* gene polymorphisms in pulmonary tuberculosis

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ABSTRACT

The *CYP2R1* gene express the enzyme 25-hydroxylase, involved in the synthesis of major circulating vitamin D metabolite 25-hydroxyvitaminD [25(OH)D]. *CYP2R1* gene variants have been reported to be associated with altered 25(OH)D level and associated with the development of active disease including tuberculosis (TB). The aim of the present study was to understand the association of rs10741657 (G/A) and rs2060793(A/G) *CYP2R1* gene polymorphisms with tuberculosis susceptibility/protection in 104 Healthy controls (HCs) and 105 pulmonary tuberculosis (PTB) patients and to understand the influence of gene variants on 25(OH)D levels in South Indian population. Genotyping was performed by polymerase chain reaction trailed by restriction fragment length polymorphism (PCR-RFLP) method. Plasma samples were used for 25(OH)D level estimation by ELISA method. In rs10741657, under a dominant model (GG vs AG + AA), "AG" and "AA" genotypes as well as in rs2060793 under an overdominant model (GA vs GG + AA), "GA" genotype were significantly associated with protection to pulmonary tuberculosis. Based on sex, rs10741657 "AG" was significantly associated with protection and "GG" was significantly associated with susceptibility to TB in males. A sufficient vitamin D level was found with rs10741657 "AA" and "AG" genotypes and "GG" genotype associated with 81.8% of vitamin D deficiency in PTB individuals. In conclusion, rs10741657 "AG" and "AA" genotypes were associated with higher 25(OH)D levels and protection to TB. The lower 25(OH)D levels associated with rs10741657 "GG" genotype individuals may be recommended for higher vitamin D supplementation for better outcome from the disease. Further studies with large sample size are needed to confirm this study finding.

1. Introduction

Tuberculosis (TB) is a health hazard and ranks one among the top 10 causes of death.The main etiological agent responsible for this disease is *Mycobacterium tuberculosis*. Globally, 10 million people fell ill with 1.3 million TB deaths among HIV-negative people in 2018. India (27%) stands topmost among eight countries which accounted for two-third of global TB burden (Global TB, 2019). Case-control studies reported independent association of vitamin D deficiency with tuberculosis, but such studies have limitations due to them being cross-sectional in nature. However, many genetic studies done earlier supported the role of vitamin D deficiency and its association with tuberculosis susceptibility.

Abbreviations: 25(OH)D, 25-hydroxyvitaminD; AIC, Akaike information criterion; BIC, Bayesian information criterion; CAMP, Cathelicidin anti-microbial peptide; CYP27B1, vitamin D 1α-hydroxylase; CYP24A1, 24-hydroxylase; CYP2R1, 25-hydroxylase; CYP, Cytochrome-P450; EDI, Epitope diagnostics; EIA, Enzyme immunoassay; GWAS, Genome-wide association studies; HCs, Healthy controls; HIV, Human Immunodefeciency virus; NIRT, National Institute for Research in Tuberculosis; OR, Odds ratio; PCR-RFLP, polymerase chain reaction - Restriction fragment length polymorphism; PTB, Pulmonary tuberculosis; TB, Tuberculosis; TE, Tris-EDTA; VDR, Vitamin D Receptor.

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Studies with Twins reported the association of genetic factors with serum 25-hydroxyvitaminD [25(OH)D] levels (Snellman et al., 2009; Karohl et al., 2010). Several candidate gene polymorphisms such as vitamin D-binding protein (*GC*), vitamin D receptor (*VDR*), vitamin D 1α-hydroxylase (*CYP27B1*), 24-hydroxylase (*CYP24A1*), and 25-hydroxylase (*CYP2R1*) are reported to be associated with serum 25(OH)D levels (Bu et al., 2010; McGrath et al., 2010). Moreover, genome-wide association studies (GWAS) have shown that candidate gene polymorphisms to be associated with 25(OH)D levels (Ahn et al., 2010; Wang et al., 2010).

CYP2R1 gene (codes: 25-hydroxylase) belongs to cytochrome-P450 (CYP) family located on the short arm of Chromosome 11(11p15.2; http:// www.ncbi.nlm.nih.gov/ gene/120227) and involved in the synthesis of major circulating vitamin D metabolite 25(OH)D and determine vitamin D status. A study conducted on Jordanian population and among North Indian population reported that Cyp2R1 rs10741657 and rs2060793 polymorphism heterozygous genotype "GA" to be associated with vitamin D deficiency and increased disease risk (Lafi et al., 2015; Haldar et al., 2018). In contrary the study conducted in Pakistani population found no association with TB, however, they identified that female participants to be more susceptible to vitamin D deficiency due to less exposure to sun compared to males. Moreover, they reported that delayed sputum conversion is genotype independent (Junaid et al., 2015). In addition, it has been reported that DNA methylation in vitamin D pathway genes lead to the development of pulmonary tuberculosis (Wang et al., 2018).

Our earlier studies reported the association of vitamin D receptor (*VDR*) gene promoter and 3'UTR polymorphisms to be associated with pulmonary tuberculosis (Selvaraj et al., 2000; Selvaraj et al., 2004; Selvaraj et al., 2008; Harishankar et al., 2018). However, the association of vitamin D pathway gene variants with pulmonary tuberculosis has not yet been studied. Hence, we performed this study to understand whether *CYP2R1* gene polymorphisms rs10741657 (G/A) and rs2060793 (A/G) are associated with susceptibility/protection to pulmonary tuberculosis and to determine the influence of gene variants on 25(OH)D levels in South Indian population.

2. Materials and methods

2.1. Study population

Healthy Control Group consisted of individuals of college students, laboratory personnel and volunteers who are asymptomatic for TB. In our study, 104 of them were healthy control and 105 Pulmonary tuberculosis patient group were positive for pulmonary tuberculosis by X-Ray and confirmed by culture test for *Mycobacterium tuberculosis*. Both the study groups were recruited from the National Tuberculosis Research Centre (NIRT), Chennai, India. This study has been approved by the ethical committee of NIRT and written consent was obtained from all participants of the study. All the individuals in this study represent the indigenous group of the South Indian population living in and around Chennai.

2.2. Genomic DNA isolation

Genomic DNA was extracted from granulocytes by simple salting out procedure as described earlier (Miller et al., 1988). At the end, genomic DNA was dissolved in Tris-EDTA (TE) buffer (pH 8.0) and purity was checked at 260/280 nm by using Nanodrop "ND1000" spectrophotometer and stored at −80 °C until use. Genotyping was done by polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP) method for CYP2R1 rs10741657(G/A) and rs2060793(A/G) polymorphisms (Lafi et al., 2015; Haldar et al., 2018). The 25(OH)D levels were estimated from stored plasma samples.

2.3. Genotyping of rs10741657

Amplification of 288 base pairs (bp) was done by PCR using forward primer (FP): 5'-GGGAAGAGCAATGACATGGA-3′ and reverse primer (RP) 3'-GCCCTGGAAGACTCATTTTG-5′. After amplification, the PCR product was digested with 3 units of *MnlI* restriction enzyme (New England Biolabs) at 37 °C overnight in a total volume of 20 μl. The homozygous "GG" genotype gave rise to 3 bands of 151 bp, 105 bp, and 32 bp fragment size, while infrequent "AA" genotype gave rise to 2 bands of 256 bp and 32 bp fragment size and the heterozygous genotype "AG" yielded 4 bands of 256 bp, 151 bp, 105 bp and 32 bp fragment size (Lafi et al., 2015).

2.4. Genotyping of rs2060793

The PCR product 820 bp was amplified using FP:5'-TTCTAGAGGCTGCCCACATTCCTT-3′ and RP: 3'-GTTGCAAAGGCAGGGTTGATCT −5′ and digested with 3 U of *HinfI* restriction enzyme (New England Biolabs) at 37 °C for overnight incubation. The homozygous "GG" genotype gave rise to 3 bands of 645 bp, 102 bp, and 73 bp fragment size, while infrequent "AA" genotype gave rise to 2 bands of 747 bp and 73 bp and the heterozygous genotype "GA" yielded 4 bands of 747 bp, 645 bp, 102 bp and 73 bp size (Haldar et al., 2018).

2.5. 25(OH)D level estimation

Epitope diagnostics (EDI), total 25(OH) vitamin D enzyme immunoassay (EIA) kit (EDI, San Diego, CA 92121, USA) was used to estimate plasma 25(OH)D levels as per protocol. The detection range of the kit is 0–150 ng/ml. The rs10741657 (G/A) and rs2060793 (A/G) gene variants were correlated with plasma 25(OH)D levels.

2.6. Statistical analysis

The allele, genotype, and haplotype frequencies were predicted using the SNP stats software. For associations involving genotypes, *p*-values with an odds ratio for gender and age were calculated by logistic regression under codominant, dominant, recessive and overdominant models. Pearson χ^2 test was used to check if the genotype frequencies fall under Hardy–Weinberg equilibrium. Odds ratio with 95% confidence intervals and the *p*-value table with Yates correction were estimated using Epi info version 6.04 (Sole et al., 2006). Based on lowest value of Akaike information criterion (AIC) and Bayesian information criterion (BIC), dominant model for rs10741657 and overdominant model for rs2060793 polymorphism was selected. The 25(OH)D levels between different genotypes in the same group was analyzed by paired "t" test and different group by independent "t" test. A p-value ≤ 0.05 was considered statistically significant.

3. Results

The genotype frequencies were under Hardy Weinberg equilibrium in both the polymorphisms.

3.1. rs10741657 (G/A) and rs2060793 (A/G) polymorphism

In rs10741657, the major allele 'G' was present among 59% in HCs and 71% in TB patients whereas 'A' allele was found 41% in HCs and 29% in patients. Under the dominant model (GG vs AG + AA), "AG" and "AA" genotypes were significantly associated with protection to pulmonary tuberculosis [OR: 0.37(0.20–0.70); $p = 0.0017$] (Table 1).

In rs2060793, the major allele 'G' was present among 58% in HCs and 65% in TB patients. Allele 'A' was found in 42% of HCs and 35% in patients. Under overdominant model (GA vs GG + AA), 'GA' genotype associated significantly with protection to pulmonary tuberculosis [OR: 0.47(0.24–0.94); $p = 0.031$] (Table 2).

Table 1
Allele and Genotype frequencies of Cyp2r1 rs10741657 polymorphism and its association with vitamin D status in HCs and PTB patients.

Test of Analysis	Allele/ Genotypes	Allele/Genotype frequencies		Odds ratio (95%CI)	p-value
		HCs	PTB		
Allele	G	0.59 (122)[$]	0.71 (150)[$]		
	A	0.41 (86)[$]	0.29 (60)[$]		
Genotype Association		**n=104**	**n=105**		
	GG	0.30(31)	0.51(54)	1	
	AG	0.58(60)	0.40(42)	**0.37 (0.20-0.70)[#]**	**0.0017[#]**
	AA	0.12(13)	0.09(09)		
Gender Association	**Female**	**n=52**	**n=24**		
	GG	0.33(17)	0.58(14)	2.88 (0.95-8.85)	0.062
	AG	0.52(27)	0.29(07)	0.38 (0.12-1.19)	NS
	AA	0.15(08)	0.13(03)	0.79 (0.15-3.77)	NS
	Male	**n=52**	**n=81**		
	GG	0.27(14)	0.50(40)	**2.65 (1.17-6.03)**	**0.017**
	AG	0.63(33)	0.43(35)	**0.44 (0.20-0.95)**	**0.035**
	AA	0.10(05)	0.07(06)	0.75 (0.19-3.04)	NS
25(OH)D Status	**Genotypes**	**25(OH)D Levels <20ng/ml**	**>20ng/ml**	**Odds ratio (95%CI)**	**p-value**
HCs		**n=11**	**n=14**		
	GG	0.273(3)	0.0(0)	UD*	0.072
	AG	0.545(6)	0.786 (11)	0.33 (0.04-2.47)	0.389
	AA	0.182(2)	0.214(3)	0.81 (0.07-8.32)	1
PTB		**n=11**	**n=9**		
	GG	0.818(9)	0.0(0)	UD*	**0.0003**
	AG	0.182(2)	0.667(6)	0.11 (0.01-1.21)	0.065
	AA	0.0(0)	0.333(3)	0.00(0.0-1.75)	0.074

Based on dominant model (GG vs AG + AA), age and sex adjusted odds ratio value and *p*-value mentioned. n represents number of individuals studied. * Undefined; $ Numbers in parenthesis denotes allelic counts. In Genotype frequencies, numbers in parenthesis denotes number of individuals positive for that genotype. Among gender and 25(OH)D status, Epi Info was used to derive *p*-value with Yates correction and odds ratios (OR) with 95% confidence intervals.

3.2. Cyp2R1 polymorphisms among male and female

Among male and female individuals, age-adjusted difference between genotype frequencies based on sex, were determined in HCs and PTB patients.

In rs10741657 polymorphism, the males with heterozygous genotype "AG" significantly associated with TB protection [OR; 0.44 (0.20–0.95; $p = 0.035$). Moreover, in males, the homozygous genotype "GG" associated with TB susceptibility [OR; 2.65(1.17–6.03; $p = 0.017$), while a trend towards susceptibility was observed in females [OR; 2.88 (0.95–8.85); $p = 0.062$] (Table 1).

In rs2060793, no significant association was observed with TB. However, in "GG" genotype odds ratio was associated with susceptibility in female [OR: 2.5(0.69–9.22) and male [OR: 1.69(0.66–4.39) individuals. In addition, a protective odds ratio was found with "GA" genotype [Male: 0.58(0.23–1.44); female: 0.31 (0.08–1.21) (Table 2).

When the genotype frequencies was analyzed for heterogeneity between male and female, it revealed that males were associated with more susceptibility compared with females in both the polymorphisms (OR > 2).

Table 2
Allele and Genotype frequencies of Cyp2r1 rs2060793 polymorphism and its association with vitamin D status in HCs and PTB patients.

Test of Analysis	Allele/ Genotypes	Allele/Genotype frequencies		Odds ratio (95%CI)	p-value
		HCs	PTB		
Allele	G	0.58 (83)[$]	0.65 (102)[$]		
	A	0.42 (61)[$]	0.35 (54)[$]		
Genotype Association		**n=72**	**n=78**		
	GG	0.31(22)	0.46(36)		
	GA	0.54(39)	0.39(30)	**0.47 (0.24-0.94)[@]**	**0.031[@]**
	AA	0.15(11)	0.15(12)		
Gender Association	**Female**	**n=35**	**n=20**		
	GG	0.29(10)	0.50(10)	2.50 (0.69-9.22)	
	GA	0.51(18)	0.25(05)	0.31 (0.08-1.21)	NS
	AA	0.20(07)	0.25(05)	1.33 (0.30-5.87)	
	Male	**n=37**	**n=58**		
	GG	0.32(12)	0.45(26)	1.69 (0.66-4.39)	NS
	GA	0.57(21)	0.43(25)	0.58 (0.23-1.44)	
	AA	0.11(04)	0.12(07)	1.13 (0.27-5.06)	
25(OH)D Status	**Genotypes**	**25(OH)D Levels <20ng/ml**	**>20ng/ml**	**Odds ratio (95%CI)**	**p-value**
HCs		**n=7**	**n=6**		
	GG	0.0 (0)	0.500 (3)	0.00 (0.0-1.78)	0.070
	GA	0.857 (6)	0.500 (3)	6.00 (0.28-246)	0.266
	AA	0.143 (1)	0.0 (0)	UD*	1
PTB		**n=9**	**n=11**		
	GG	0.445 (4)	0.454 (5)	0.96 (0.11-8.07)	1
	GA	0.333 (3)	0.364 (4)	0.88 (0.09-8.00)	1
	AA	0.222 (2)	0.182 (2)	1.29 (0.09-18.20)	1

@ Based on overdominant model (GA vs GG + AA), age and sex adjusted odds ratio value and p-value mentioned. n represents number of individuals studied. * Undefined; $ Numbers in parenthesis denotes allelic counts. In Genotype frequencies, numbers in parenthesis denotes number of individuals positive for that genotype. Among gender and 25(OH)D status, Epi Info was used to derive p-value with Yates correction and odds ratios (OR) with 95% confidence intervals.

3.3. Haplotype frequency

Among haplotypes such as GG, AA, GA, and AG no significant result was found with haplotype frequencies between HCs and PTB patients. Among those with "GG" frequency was found higher (HCs: 53.8%; PTB: 63.3%) followed by "AA" (HCs: 35.5%; PTB: 23%), "GA" (HCs: 6.2%; PTB: 8.5%) and "AG" (HCs: 4.5%; PTB: 5.2%). However, sex within haplotypes revealed "GA" and "GG" haplotypes to be associated with TB susceptibility in males [GG: OR; 3.21 (0.92–11.23); GA: OR; 4.67 (0.32–67.34)] compared with females.

3.4. 25(OH)D levels among variant genotypes of Cyp2R1 polymorphisms

Among genotypes of rs10741657 polymorphism, the higher 25(OH) level was found with "AA" genotype and the lower level was associated with "GG" genotype in HCs and PTB patients. When the 25(OH) level was compared between variant genotypes, significantly higher level was found with "AG" and "AA" genotypes compared with "GG" genotype ("GG vs "AG": HCs, p=0.026; PTB, p<0.0001; "GG" vs "AA": HCs, p=0.036; PTB, p=0.0091). In addition, a significantly higher level was observed with "AA" genotype compared with "AG" genotype of PTB patients ("AG" vs "AA"; $p = 0.012$) (Fig 1A).

In variant genotypes of rs2060793 polymorphism, higher 25(OH) level was found with "AA" genotype and lesser level was observed with "GG" genotype of PTB patients. No significant difference was observed in 25(OH) level among genotypes in HCs and PTB patients (Fig 1B).

3.5. Cyp2R1 gene variants among deficient and sufficient 25(OH)D levels

The rs10741657 and rs2060793 polymorphic gene variants were compared between <20 ng/ml (deficient) and > 20 ng/ml (sufficient) vitamin D status. In rs10741657 polymorphism, individuals with "AG" (OR: 0.11 (0.01–1.21), $p = 0.065$) and "AA" genotypes (OR: 0.00 (0.0–1.75), $p = 0.074$) were associated with sufficient 25(OH)D level in PTB patients ("AG": 66.7%; "AA": 33.3%). Moreover, "GG" genotype was significantly associated with deficient 25(OH)D level (81.8%) in PTB patients ($p = 0.0003$) while a similar trend was observed in HCs ($p = 0.072$). In addition, none of the individuals were positive for sufficient level in "GG" genotype (Table 1). Hence, we observed undefined odds ratio in both the study groups. However, there no significant association found with rs2060793 gene variants with deficient and sufficient 25 (OH)D level in HCs and PTB patients (Table 2).

4. Discussion

Nutritional deficiency, particularly vitamin D is reported to be associated with TB in various ethnic populations (Gibney et al., 2008; Nnoaham and Clarke, 2008). Several studies such as twin, family-based studies, candidate gene analysis and genomic-wide association studies (GWAS) confirmed the association of genetic factors with 25(OH)D levels and accounts from 23 to 80% (Snellman et al., 2009; Karohl et al., 2010; Shea et al., 2009; Bu et al., 2010; McGrath et al., 2010; Ahn et al., 2010; Wang et al., 2010). In addition, ethnic variations also have been reported (Signorello et al., 2011). Polymorphisms in *CYP2R1* gene has been reported to be associated with 25(OH)D deficiency (Ahn et al., 2010; Lafi et al., 2015). Since the association of *CYP2R1* gene variants with TB has not yet studied in our population, we attempted to understand if rs10741657(G/A) and rs2060793(A/G) polymorphisms were associated with pulmonary tuberculosis and to determine the influence of *CYP2R1* polymorphic variants on 25(OH)D levels in our population.

Our study revealed that in rs10741657(G/A) polymorphism, a significant protective association was found with "AG" and "AA" genotypes under a dominant model (GG vs AG + AA) with pulmonary tuberculosis [OR: 0.37(0.20–0.70); $p = 0.0017$] (Table 1). A similar protective association was found with heterozygous 'GA' genotype of rs2060793 (A/G) polymorphism under overdominant model (GA vs GG + AA) [OR: 0.47(0.24–0.94); $p = 0.031$] (Table 2). Based on gender, a protective association was observed with rs10741657 "AG" genotype in males [OR; 0.44(0.20–0.95; $p = 0.035$). Moreover, "GG" genotype was associated with risk for TB in male [OR; 2.65(1.17–6.03); $p = 0.017$] and similar trend was found in female [OR; 2.88(0.95–8.85); $p = 0.062$] (Table 1). Haplotypes within sex revealed that "GA" [OR; 4.67 (0.32–67.34)] and "GG" [OR; 3.21 (0.92–11.23)] to be associated with TB susceptibility in males compared with females. In the study conducted among Jordanian and Denmark population, a similar protective association was found with "AG" and "AA" genotypes of rs10741657 polymorphism and a risk was associated with "GG" genotype (Lafi et al., 2015; Nissen et al., 2014). Longitudinal and Case-control studies conducted in Lahore reported no association in rs2060793 (A/G) polymorphic variants with TB, however the similar protective trend was found with "GA" genotype

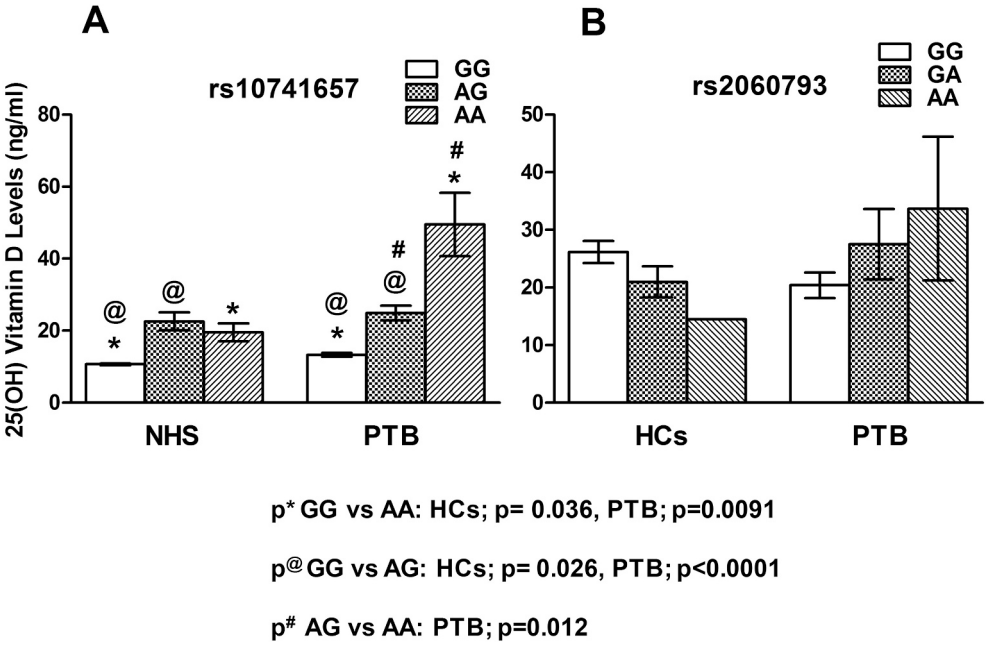

Fig. 1. Vitamin D levels among gene variants in HCs and PTB patients A. 25 (OH)D levels among rs70141657 gene variants in HCs (GG, n = 3; AG, $n = 17$; AA, n = 5) and PTB (GG, $n = 9$; AG, n = 8; AA, n = 3) patients. Results are expressed as mean ± SD. p value analyzed by paired't' test among same group. In HCs: $p^* = 0.036$; $p^@ = 0.026$. In PTB: $p^* = 0.0091$; $p^@ < 0.0001$ and $p^\# = 0.012$. **B.** 25(OH)D levels among rs2060793 gene variants in HCs (GG, n = 3; GA, n = 9; AA, n = 1) and PTB (GG, n = 9; GA, $n = 7$; AA, $n = 4$) patients.

(Junaid et al., 2015; Junaid et al., 2016).

Our earlier studies revealed that vitamin D receptor (VDR) gene variants to be associated with vitamin D levels and upregulated the expression of antimicrobial peptide cathelicidin (CAMP) which enhance the intracellular killing of *M. tuberculosis* in macrophages (Selvaraj et al., 2009). Similarly, we studied the influence of *CYP2R1* gene variants on vitamin D level in HCs and PTB patients. In both study groups, a significantly higher 25(OH)D level was found with "AA" and "AG" genotypes compared with "GG" genotype in rs10741657(G/A) polymorphism while no significant difference was found with rs2060793(A/G) polymorphism. Based on the analysis between vitamin D status being below (deficient) or above (sufficient) 20 ng/ml (Table 1), In rs10741657 "GG" genotype 81.8% of PTB individuals significantly ($p = 0.0003$) associated with vitamin D deficiency (<20 ng/ml) and a similar trend was observed with 27.3% in HCs ($p = 0.072$). None of the individuals were found with sufficient level and the odds ratio was found to be undefined in both the study groups. Whereas, among rs10741657 "AG" genotype, 66.7% of PTB individuals were found to have sufficient 25(OH)D levels and 18.2% were found to have 25(OH)D deficiency (Odds ratio [OR]: 0.11(0.01–1.21); $p = 0.065$). In rs10741657 "AA" genotype, 33.3% of the PTB individuals were found with sufficient 25 (OH)D levels and none of them had deficient level (OR:0.0[0.0–1.75]; $p = 0.0740$). A similar trend was found in HCs but the difference not found significant (Table 1). Similar to our finding, rs10741657 "AA" and "AG" genotypes were associated with increased 25(OH)D level in Jordanian population (Lafi et al., 2015), in a genome-wide association study conducted in Chinese population (Wang et al., 2010) and from a study reported from a German population (Ramos-Lopez et al., 2007). The longitudinal study conducted in Lahore revealed that genetic variants of rs2060793 (A/G) polymorphism didn't associate with vitamin D status as found in our study (Junaid et al., 2015). Moreover, they that response to anti-tuberculosis treatment was not associated with rs2060793 gene variants and that vitamin D deficiency was observed in 55% of "GG" genotype, 58% and 50% among "GA" and "AA" genotype individuals. In addition, they reported that delayed sputum conversion was associated with vitamin D deficiency in a genotype independent mode (Junaid et al., 2015).

Studies reported the control of gene expression by epigenetic which involves DNA methylation of cytosine in a CpG dinucleotide (Guastafierro et al., 2017). It has been shown that hypermethylation in cytochrome 450 gene promoter leads to suppression of vitamin D levels (Zhu et al., 2013). A study identified methylation pattern in the promoter region of vitamin D pathway genes, including CYP2R1 and its association with lower vitamin D levels. The results identified 55 different methylated CpG sites between cases and controls. Moreover, among 23 treatment outcome related CpG sites 5.9% associated with CYP2R1 gene (Wang et al., 2018). Another study reported that CYP2R1 baseline methylation inversely correlated with 25(OH)D levels and hyper DNA methylation needed higher vitamin D supplementation to optimize the level (Zhou et al., 2014). The individuals with lower 25(OH)D level in rs10741657 "GG" genotype in this study might have hypermethylation in CpG sites compared with "AA" and "AG" genotype individuals. The observational studies reported that vitamin D deficiency associated with poor response to anti-TB therapy. In a North Indian study conducted in 354 multi-drug resistant TB patients found negative correlation between 25(OH)D level and sputum conversion time (Rathored et al., 2012). A similar result was found with Japanese study conducted in 34 drug-sensitive cases (Sato et al., 2012). Moreover, vitamin D in-sufficiency is reported to be associated with treatment relapse in a cohort of TB patients in Tanzania (Mehta et al., 2013). In addition, studies using higher dosage of vitamin D are found to be associated with accelerated sputum conversion in around 14 days (Nursyam et al., 2006; Coussens et al., 2012). However, inconsistency was observed with randomised-controlled trails compared with observational studies due to improper vitamin D dosing regimens and small sample size. The above studies suggest that the deficient 25(OH)D level found with rs10741657 "GG" genotype might be associated with hypermethylation which could lead to the development of disease severity and delayed response to anti-TB treatment. This study has limitations due to ethnic variation and small sample size, which could be a predominant limiting factor to hinder the detection of minor associations that needs to be resolved by expanding the study with larger sample size.

In conclusion, rs10741657 "AG" and "AA" genotypes were significantly associated with protection to TB under a dominant model (GG vs AG + AA). A similar protective association was found with heterozygous rs2060793 'GA' genotype under overdominant model (GA vs GG + AA). Based on gender, males were associated with more susceptibility compared with females. In rs10741657 "GG" genotype was significantly associated with TB risk in male and similar trend was found in females. A sufficient vitamin D level was found with rs10741657 "AA" and "AG" genotypes while in "GG" genotype, 81.8% of PTB individuals significantly were associated with vitamin D deficiency and such individuals may be recommended for higher vitamin D supplementation to optimize the level for a better outcome from the disease. Further studies with larger sample size are needed to confirm the association of these gene variants with vitamin D level and its association with TB outcome.

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Ethical policy

The study was approved by the Institutional Ethics Committee NIRT. Reference no. 1/12/108/JMI/IEC/2016. Informed consent obtained from all participants.

Credit author statement

Dr.MH: Concept and design, review of literature, acquisition of data, analysis and interpretation of data, drafting and revising the manuscript. Mrs.SP: Acquisition and analysis of Data, analysis revision of draft for manuscript. Ms. MS, Mr. RR & Mr.VA: Data Acquisition. Dr.CP: Analysis and interpretation of statistical data. Dr.VB: Acquisition of data through Clinical diagnosis and Patient recruitment. Obtained consent from patients. Dr.PU: Revision of the manuscript. Dr.SPT & Dr.UD: Resource and Revision of the manuscript. Dr.PS: Conception of idea, design and revision of the manuscript. Dr.BR: Conception of idea, design, data analysis and interpretation of data, drafting and revision of the manuscript.

Declaration of Competing Interest

All authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Ahn, J., Yu, K., Stolzenberg-Solomon, R., Simon, K.C., McCullough, M.L., et al., 2010. Genome-wide association study of circulating vitamin D levels. Hum. Mol. Genet. 19, 2739–2745.

Bu, F.X., Armas, L., Lappe, J., Zhou, Y., Gao, G., et al., 2010. Comprehensive association analysis of nine candidate genes with serum 25-hydroxy vitamin D levels among healthy Caucasian subjects. Hum. Genet. 128, 549–556.

Coussens, A.K., Wilkinson, R.J., Hanifa, Y., Nikolayevskyy, V., Elkington, P.T., Islam, K., et al., 2012. Vitamin D accelerates resolution of inflammatory responses during tuberculosis treatment. Proc. Natl. Acad. Sci. U. S. A. 109 (38), 15449–15454.
Gibney, K.B., MacGregor, L., Leder, K., et al., 2008. Vitamin D deficiency is associated with tuberculosis and latent tuberculosis infection in immigrants from sub-Saharan Africa. Clin. Infect. Dis. 46, 443–446.
Global TB, 2019. report.
Guastafierro, T., Bacalini, M.G., Marcoccia, A., Gentilini, D., Pisoni, S., Di Blasio, A.M., Corsi, A., Franceschi, C., Raimondo, D., Spano, A., et al., 2017. Genome-wide DNA methylation analysis in blood cells from patients with Werner syndrome. Clin. Epigenetics 9, 92.
Haldar, Deepa, Agrawal, Nitin, Patel, Seema, Kambale, Pankaj Ramrao, Arora, Kanchan, Sharma, Aditi, Tripathi, Manish, Batra, Aruna, Kabi, Bhaskar C., 2018. Association of VDBP and CYP2R1 gene polymorphisms with vitamin D status in women with polycystic ovarian syndrome: a north Indian study. Eur. J. of Nut. 57, 703–711.
Harishankar, M., Selvaraj, P., Bethunaickan, R., 2018. Influence of genetic polymorphism towards pulmonary tuberculosis susceptibility. Front. Med. (Lausanne). 16 (5), 213.
Junaid, K., Rehman, A., Saeed, T., Jolliffe, D.A., Wood, K., Martineau, A.R., 2015. Genotypeindependent association between profound vitamin D deficiency and delayed sputum smear conversion in pulmonary tuberculosis. BMC Infect. Dis. 15, 275.
Junaid, K., Rehman, A., Jolliffe, D.A., Saeed, T., Wood, K., Martineau, A.R., 2016. Vitamin D deficiency associates with susceptibility to tuberculosis in Pakistan, but polymorphisms in VDR, DBP and CYP2R1 do not. BMC. Pulm. Med. 16 (1), 73.
Karohl, C., Su, S., Kumari, M., Tangpricha, V., Veledar, E., et al., 2010. Heritability and seasonal variability of vitamin D concentrations in male twins. Am. J. Clin. Nutr. 92, 1393–1398.
Lafi, Z.M., Irshaid, Y.M., El-Khateeb, M., Ajlouni, K.M., Hyassat, D., 2015. Association of rs7041 and rs4588 polymorphisms of the vitamin D binding protein and the rs10741657 polymorphism of CYP2R1 with vitamin D status among Jordanian patients. Genet. Test. Mol. Biomarkers. 19 (11), 629–636.
McGrath, J.J., Saha, S., Burne, T.H., Eyles, D.W., 2010. A systematic review of the association between common single nucleotide polymorphisms and 25-hydroxyvitamin D concentrations. J. Steroid Biochem. Mol. Biol. 121, 471–477.
Mehta, S., Mugusi, F.M., Bosch, R.J., Aboud, S., Urassa, W., Villamor, E., et al., 2013. Vitamin D status and TB treatment outcomes in adult patients in Tanzania: a cohort study. BMJ Open 3 (11), e003703.
Miller, S.A., Dykes, D.D., Polesky, H.F., 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 16, 1215.
Nissen, J., Rasmussen, L.B., Ravn-Haren, G., Andersen, E.W., Hansen, B., Andersen, R., Mejborn, H., Madsen, K.H., Vogel, U., 2014. Common variants in CYP2R1 and GC genes predict vitamin D concentrations in healthy Danish children and adults. PLoS One 9 (2), e89907.
Nnoaham, K.E., Clarke, A., 2008. Low serum vitamin D levels and tuberculosis: a systematic review and meta-analysis. Int. J. Epidemiol. 37, 113–119.
Nursyam, E.W., Amin, Z., Rumende, C.M., 2006. The effect of vitamin D as supplementary treatment in patients with moderately advanced pulmonary tuberculous lesion. Acta. Med. Indones. 38 (1), 3–5.
Ramos-Lopez, E., Brück P, Jansen T, et al., 2007. CYP2R1 (vitamin D 25-hydroxylase) gene is associated with susceptibility to type 1 diabetes and vitamin D levels in Germans. Diabetes Metab. Res. Rev. 23, 631–636.
Rathored, J., Sharma, S.K., Singh, B., Banavaliker, J.N., Sreenivas, V., Srivastava, A.K., et al., 2012. Risk and outcome of multidrug-resistant tuberculosis: vitamin D receptor polymorphisms and serum 25(OH)D. Int. J. Tuberc. Lung. Dis. 16 (11), 1522–1528.
Sato, S., Tanino, Y., Saito, J., Nikaido, T., Inokoshi, Y., Fukuhara, A., et al., 2012. Relationship between 25-hydroxyvitamin D levels and treatment course of pulmonary tuberculosis. Respir. Investig. 50 (2), 40–45.
Selvaraj, P., Narayanan, P.R., Reetha, A.M., 2000. Association of vitamin D receptor genotypes with the susceptibility to pulmonary tuberculosis in female patients & resistance in female contacts. Indian J. Med. Res. 111, 172–179.
Selvaraj, P., Chandra, G., Jawahar, M.S., Rani, M.V., Rajeshwari, D.N., Narayanan, P.R., 2004. Regulatory role of vitamin D receptor gene variants of Bsm I, Apa I, Taq I, and Fok I polymorphisms on macrophage phagocytosis and lymphoproliferative response to mycobacterium tuberculosis antigen in pulmonary tuberculosis. J. Clin. Immunol. 24 (5), 523–532.
Selvaraj, P., Alagarasu, K., Harishankar, M., Vidyarani, M., Narayanan, P.R., 2008. Regulatory region polymorphisms of vitamin D receptor gene in pulmonary tuberculosis patients and normal healthy subjects of South India. Int. J. Immunogenet. 35 (3), 251–254.
Selvaraj, P., Prabhu Anand, S., Harishankar, M., et al., 2009. Plasma 1,25 dihydroxy vitamin D3 level and expression of vitamin d receptor and cathelicidin in pulmonary tuberculosis. J. Clin. Immunol. 29, 470–478.
Shea, M.K., Benjamin, E.J., Dupuis, J., Massaro, J.M., Jacques, P.F., et al., 2009. Genetic and non-genetic correlates of vitamins K and D. Eur. J. Clin. Nutr. 63, 458–464.
Signorello, L.B., Shi, J., Cai, Q., Zheng, W., Williams, S.M., et al., 2011. Common variation in vitamin D pathway genes predicts circulating 25-hydroxyvitamin D levels among African Americans. PLoS One 6, e28623.
Snellman, G., Melhus, H., Gedeborg, R., Olofsson, S., Wolk, A., et al., 2009. Seasonal genetic influence on serum 25-hydroxyvitamin D levels: a twin study. PLoS One 4, e7747.
Sole, X., Guino, E., Valls, J., et al., 2006. SNPStats: a web tool for the analysis of association studies. Bioinformatics. 22, 1928–1929.
Wang, T.J., Zhang, F., Richards, J.B., Kestenbaum, B., van Meurs, J.B., et al., 2010. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet 376, 180–188.
Wang, M., Kong, W., He, B., Li, Z., Song, H., Shi, P., Wang, J., 2018. Vitamin D and the promoter methylation of its metabolic pathway genes in association with the risk and prognosis of tuberculosis. Clin. Epigenetics 10 (1), 118.
Zhou, Y., Zhao, L.J., Xu, X., Ye, A., Travers-Gustafson, D., Zhou, B., Wang, H.W., Zhang, W., Lee Hamm, L., Deng, H.W., Recker, R.R., Lappe, J.M., 2014. DNA methylation levels of CYP2R1 and CYP24A1 predict vitamin D response variation. J. Steroid Biochem. Mol. Biol 144, 207–214.
Zhu, H., Wang, X., Shi, H., Su, S., Harshfield, G.A., Gutin, B., Snieder, H., Dong, Y.A., 2013. Genome-wide methylation study of severe vitamin D deficiency in African American adolescents. J. Pediatr. 162 (5), 1004–1009 (e1001).