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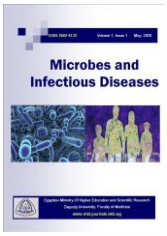

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Originalarticle

Linking 5'-promoter region of Cdx2 VDR polymorphism and serum vitamin D association in female genital tuberculosis

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ABSTRACT

Background: Globally, genital tuberculosis (GTB) is a substantial cause of female infertility. The vitamin D receptor (VDR), a member of the nuclear receptor superfamily, mediates the immunological function of vitamin D3, activates macrophages, and vitamin D deficiency has been linked to female genital tuberculosis (FGTB) risk. **Objective**: The aim of this study was to assess the relationship between risk of FGTB and the vitamin D receptor gene polymorphism (Cdx2) and serum 25 (OH) D3 level.**Methods**: 150 confirmed FGTB cases and 150 healthy controls were recruited. Serum 25 (OH) D3 level was measured by ELISA. Genomic DNA was extracted and the genotyping of VDR-Cdx2 polymorphism was performed by Tetra-primer amplification refractory mutation system (T-ARMS-PCR).**Results**: Serum 25(OH) D3levels were significantly lower among FGTB patients. The frequency of A allele was 68% in FGTB and 48.6% in control; A allele was significantly associated with increased risk of FGTB [OR = 2.24; 95 % confidence interval CI=1.26-1.81; $p< 0.0001$].However, the frequency of G allele was 32% in FGTB and 51.3% in control; G allele did not show significant risk of FGTB [OR = 1.10; 95 % confidence interval CI=0.78-1.44; $p = 0.67$]. A significant association was found between VDR Cdx-2 AA ($p<0.001$) genotype and Serum 25(OH) D3level.**Conclusion**: Genotype frequencies of VDR gene polymorphism and serum vitamin D level were found to have significant association leads. VDR dysfunction could increase FGTB risk. Understanding the synergism between VDR polymorphism and serum vitamin D in FGTB will be important to identifying the new prognostic tool and target for therapy in serum vitamin D deficient individuals. .

Introduction

The second most common infectious killer after COVID-19 is tuberculosis (TB), which ranks as the 13th leading cause of death.The Global tuberculosis report (WHO) estimates that 10.6 million individuals will have contracted the disease worldwide by 2022, resulting in 1.6 million deaths [1]. India had a continuous increase in tuberculosis cases in 2021, which was 19% higher than in 2020 [2]. The two most common forms of TB are pulmonary tuberculosis and extra-pulmonary tuberculosis (EPTB). Extrapulmonary tuberculosis (EPTB) accounts for around 20–30% of all active tuberculosis cases. The prevalence of genital tuberculosis in India has recently been found to range from 45.1 cases per 100,000 women in

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community-based research in the Andaman Islands to 48.5 % among infertile women in North India. Genital tuberculosis is a common form of EPTB, responsible for 27 % of all EPTB cases transmitted through hematogenous or lymphatic routes [3]. Genital tuberculosis (GTB) in females is usually asymptomatic, though it may cause fever, menstrual irregularities, chronic pelvic inflammation, and permanent damage to the female genital tract in advanced stages [4]. Despite recent medical advancements and the availability of advanced investigation techniques, diagnostic challenges still exist as a result of FGTB's paucibacillary nature. Thus, early subclinical stage detection and treatment can prevent lasting damage with a successful pregnancy outcome. Vitamin D found in two forms, D2 and D3. Vitamin D3 activates the body's immune system against a variety of infectious pathogens, including *M. tuberculosis*, and plays a significant role in human innate immunity by activating monocytes [5]. Vitamin D regulates its activity by binding with the Vitamin D receptor (VDR), a nuclear hormone receptor family located on the long (q) arm of chromosome 12 at position 13.11 [6]. It has been shown that vitamin D deficiency (serum 25(OH)D3 level <10 ng/mL) and insufficiency (serum 25(OH)D3 level <20 ng/mL)are associated with a higher risk of active TB[7]. Genetic susceptibility has been considered one of the most important factors in individual TB risk [8]. Several population-based case control studies have linked allelic variations of the VDR gene, which may alter VDR function and enhance susceptibility to immune mediated disorders, to tuberculosis. Nevertheless, the findings of these studies have produced conflicting conclusions [9–10]. More than 200 VDR polymorphisms have been identified in different individuals, including the 3′ untranslated region (3′UTR) polymorphisms ApaI (T→G) in intron 8, TaqI(T→C) in exon 9, and the start codon polymorphism FokI, which have been intensively investigated in association with TB [11–13]. In the VDR 5'-promoter region, two SNPs were identified: a G/A substitution in the Cdx2 binding site (1e-G-1739A, rs11568820) and an A/G substitution in the GATA binding site (1a-A-1012G, rs4516035) [14–16]. The VDR-Cdx2 polymorphism (1e-G-1739A) may influence vitamin D regulation during calcium absorption [17]. While another (rs11568820) is a G to A base substitution that affects the Cdx2 binding site and may be thought to influence the functional activity of the VDR receptor, the (1a-A-1012G) polymorphism allows naive T cells to polarize Th2 cells [18]. Numerous studies have also found a link between 3' untranslated region (UTR) polymorphisms and tuberculosis [19–22]. A significant risk factor for the development of TB has been identified in addition to the aforementioned genetic predisposition: hypovitaminosis. It is yet unknown how these host genetic variables, particularly female genital tuberculosis, affect the course of EPTB and require more research. Because, genital tuberculosis and pulmonary TB likely have different underlying pathophysiology, unraveling the mechanisms leads to a better understanding of the pathogenesis of FGTB and may help in the development of novel strategies for prevention and clinical management. To the best of our knowledge, no studies have documented the association of the VDR Cdx2 gene polymorphism with FGTB. Therefore, the objective of this study was to identify the possible association of VDR Cdx2 polymorphism and serum vitamin D level as a risk for FGTB.

Methods

This was a case-control study conducted at Department of Obstetrics & Gynecology and Department of Microbiology, King George's Medical University, Lucknow, UP, India from 2020 to 2022. The ethical approval was obtained from the institutional ethical clearance committee (No: ECR/262/Inst/UP/2013/RR-19) for human research. A written informed consent form was taken from each case and control that was enrolled in the study. A total of 300 subjects (n = 150 cases and n = 150 controls) were included in this study, fulfilling the given definitive subject selection criteria for case and control. Women's age group (20–35) years, infertility primary or secondary for a period greater than one year with a positive acid fast bacilli (AFB), Lowenstein-Jensen (LJ)-culture, *Mycobacterium* Growth Indicator Tube (MGIT) culture, histopathology (HPE), and cartridge-based nucleic acid amplification test (CBNAAT) in endometrial aspiration (EA), were recruited as inclusion criteria of cases. FGTB leads to infertility so that; we recruited infertile women in this study. Controls were recruited with no history of Anti tubercular treatment, and all tested negative for TB. Women with endometriosis, polycystic ovarian syndrome (PCOS), polycystic ovaries, *Chlamydia*, gonorrhea, or any genetic disease, already on ATT or diagnosed to have active pulmonary tuberculosis or active

extrapulmonary tuberculosis in regions other than the female genital tract, were excluded. The sample size was calculated with 80% of power.

Detection of FGTB

Premenstrual endometrial aspiration was taken on day 21 from all the recruited infertile women for the confirmation of *Mycobacterium tuberculosis* by conventional acid fast bacilli (AFB), Lowenstein-Jensen (LJ)-culture, *Mycobacterium* Growth Indicator Tube (MGIT) culture,histopathology (HPE)and molecular test cartridge-based nucleic acid amplification test (CBNAAT) for the detection of genital tuberculosis.

Serum vitamin D level estimation

Human blood sample (2 ml) was collected in EDTA vial (anticoagulant) from each subject. The serum was immediately separated by centrifugation at 2,500 rpm for 15 minutes at 4°Cand stored in 20°C. The isolated serum was further used for the quantification of 25(OH) D3 level. Serum vitamin D level was estimated by a 25-OH vitamin D ELISA kit (Eagle Bio-Sciences cat#VID31-K01) using an ELISA (Bio-Rad) iMarkMicroplate reader. The steps were followed precisely as instructed by the manufacturer. Each test was performed in duplicate, with the mean absorbance calculated from the average of two wells normalized to a zero calibrator well. The absorbance was measured using a dual wavelength reference filter with 570 and 650 nm wavelengths. Vitamin D levels were reported in nanograms per milliliter. Serum 25(OH)D3 levels are classified as deficient if they are <10 ng/ml, insufficient if they are <20 ng/mL, and sufficient if they are greater than ≥30 ng/ml.

Vitamin D receptor (VDR) genotyping

Venous blood (5 ml) was drawn from each subject at the time of recruitment and collected in tubes with ethylenediaminetetraacetic acid (EDTA). The plasma was immediately separated by centrifugation at 3,500 rpm for 15 minutes at 4°C. The cell pack was stored at -80°C until DNA extraction. Genomic DNA was extracted using the phenol-chloroform method, and purified DNA was stored at -20°C. The Cdx-2 (rs11568820) genotypes were determined by a tetra-primer set using amplification refractory mutation system polymerase chain reaction (T-ARMS-PCR).

The forward (**F**) (5-AGGATAGAGAAAATAATAGAAAACATT-3) and Reverse (**R**) (5AACCCATAATAAGAAATAAGTTTTTAC-3) primers amplified the G allele, with a product size of 110 bp. The Forward (F) (5-TCCTGAGTAAACTAGGTCACAA-3) and Reverse (R) (5-ACGTTAAGTTCAGAAAGATTAATTC-3)primer pairs specifically amplified the A allele, with a product size of 235 bp.A 297 bp internal control was amplified using the G-Forward and A-Reverse primers. The polymorphism undergoes with internal control responses to avoid false negative result. The PCR reaction was performed to follow the optimized protocolof BSL3 lab of Microbiology, KGMU Lucknow, with 5 uL of extracted DNA and amplification with 1X buffer, 1U Taq DNA polymerase, 200 mMdNTP, 1.5 mM $Mgcl_2$, and 10 pm of each primer in a total volume of 25 uL using GoTaqGreen Master Mix (Promega Inc., Madison, WI, USA). The PCR thermal cycler conditions were as follows: Initial denaturation: 94 °Cfor 3 min, 40 cycles of 95 °C (30s), 58 °C (45s), and 72 °C (45s), followed by a final extension of 72 °Cfor 5 min. PCR amplified products were electrophoresed with a 100 bp DNA ladder (Gene DireX, Inc.) on a 2% agarose gel and stained with ethidium bromide. PCR products were visualized using a UV Gel Doc (Bio-Rad, U.S.A.) and image lab software.

Statistical analysis

Statistical analysis of the data was performed with Graph Pad Prism version 5.0 software (La Jolla, CA, USA). Genotype and allele frequency of the VDR gene polymorphism in FGTB cases and controls were according to the Hardy Weinberg equilibrium. The difference in genotype frequencies between cases and controls was evaluated by the Pearson Chi-square test and data were subjected to student t test for two groups and one way ANOVA for more than two group to calculate significance between groups (***p<0.001, ** p<0.01,*p<0.05, NS p>0.05). The association of Cdx2 polymorphism with the risk of FGTB was determined by an unadjusted odds ratio (OR) and a 95% confidence interval (CI) was calculated by univariate binary logistic regression analysis. The p-value<0.05 was considered statistically significant.

Results

Anthropometric parameter and serum vitamin D levels

The Anthropometric parameter and serum vitamin D levels between cases and controls are summarized in **Table (1)**.There was no statistically

significant difference found in the anthropometric parameters between cases and controls. However, serum 25(OH) D3levels were (9.94±0.14) ng/ml in the FGTB case and (29.04 ± 0.14) ng/ml in controls showing statistically significant association(***p***<0.0001).

Comparison of diagnostic conventional and molecular tests

Table (2) shows the standard conventional AFB-smear, LJ culture, MGIT culture, HPE, and molecular test CB-NAAT. 11 (7.3%), 6 (4%), 4 (2.6%), 3 (2%), and 149 (99.3%) of the total 150 FGTB patients were tubercular positive. By comparing all four tests, 149 (99.3%) were found positive only with CBNAAT and negative with the other conventional tests **(Figure 1A and B)**.CB-NAAT, accredited real-time PCRs (RT-PCR), also called Gene-Xpert, have a detection limit of one bacillus/ml, diagnose TB and rifampicin resistance simultaneously within 2 hours, and are more promising tools for detection of FGTB with higher specificity; therefore, the patients found positive only with CBNAAT were taken as cases for VDR genotyping in this study.

Association of Cdx-2 gene polymorphism and FGTB

Genotype frequencies of Cdx2 conformed to the Hardy-Weinberg equilibrium both in cases and controls. Regarding the genotype and allele frequency distribution of the VDR-Cdx2 gene, GG, GA, AA, and G, A were 20, 24, 56, and 32, 68% in patients, while 30, 42.6, 27.3, and 51.3, 48.6% in controls, respectively, as shown in **Figure (2)**. Regarding the risk of developing FGTB, the analysis showed that the A allele was significantly associated with an increased risk of FGTB odds; 2.24 (1.26-1.81; 95% CI, ***p***< 0.0001while, G allele did not show any significant risk for FGTB odds; 1.10 (0.78-1.44; 95% CI, ***p*** = 0.67). The genotypes AA odds; 3.07 (1.24-2.27; 95% CI, ***p***<0.0002) and GA odds; 0.27 (0.15-0.47; 95% CI, ***p***<0.001) were significantly associated with the increased risk of FGTB; however, no significant association was observed with the GG genotype odds; 1.18 (0.75-1.62; 95% CI, ***p*** = 0.58) and the risk of FGTB.

The odds ratio for the recessive model (GG+GA vs. AA) was 3.38 (CI = 2.08–5.48, ***p*** =0.001), whereas the dominant model (AA+GA vs. GG) was 0.58 (CI = 0.34-0.99, ***p*** = 0.06)**(Table 3)**.

The above results suggest that the frequency of the GG genotype and G allele was significantly lowered, showed a protective polymorphism, and may not be associated with developing FGTB risk in patients; however, the increased frequency of the AA genotype and A allele may increase the risk of FGTB susceptibility.

Association between serum 25(OH)D3 levels and FGTB risk

Out of 150 cases, 124 were found to have vitamin D levels of <10 ng/ml, 14 had levels <20 ng/ml and 12 had ≥ 30 ng/ml, respectively.**Table (4)** showed that the FGTB patients who were vitamin D insufficient (<20 ng/ml; ***p***< 0.001) and deficient for 25(OH) D3 (<10 ng/ml; ***p***< 0.001) had an increased risk of FGTB compared to vitamin D sufficient (≥30 ng/ml; ***p*** = 0.229) patients.

Vitamin 25(OH) D3 levels and the VDR Cdx-2 gene

The relation between vitamin D levels and various genotype frequencies is depicted in **Figure (4)**. The statistically significant mean difference of serum 25(OH) D3 levels was found with AA genotype (***p***< 0.0001) in FGTB patients; however, no significant difference in vitamin D level was found in the GG and GA genotypes in FGTB patients as compared to controls **(Table5)**.

Table 1. Anthropometric parameter and serum vitamin D level in FGTB case (n=150) andcontrol (n=150).

Variables	Age (Mean±SD) (Yrs./old)	Weight (Mean ± SD) kg	BMI (kg/m2)	Serum Vitamin D (ng/ml)
FGTB	28.05 ± 3.63	53.08±0.48	22.42±0.22	9.94±0.14

Case (n=150)				
Healthy Controls (n=150)	27.25 ± 3.53	54.36 ± 0.50	23.07 ± 0.23	29.04 ± 0.14
p-value	0.054	0.067 [a]	0.046[a]	0.0001*[a]

Abbreviations:SD, standard deviation, *a calculated by t-test, * Significantly different from control (***p***<0.05).

Table 2. Correlation of conventional and molecular test in FGTB cases**.**

Conventional Test	Criteria	FGTB Cases (n=150)	Molecular Test CBNAAT		***p*** value
			Positive(n=149)	Negative (n=1)	
AFB Smear	Positive	11(7.3%)	11(99.3%)	0	
	Negative	139(92.6 %)	138(92.6%)	1 (100%)	0.001*
LJ	Positive	6(4%)	6 (4%)	0	
	Negative	144(96%)	143(95.9%)	1(100%)	0.001*
MGIT	Positive	4(2.6 %)	4(2.6 %)	0	
	Negative	146(97.3%)	145(97.3%)	1(100%)	0.001*
HPE	Positive	3(2%)	3(2%)	0	
	Negative	147(98%)	146(98%)	1(100%)	0.001*

Table 3. Genotype and allele frequency distribution of the Cdx2polymorphism among FGTB cases (n=150) and controls (n=150).

Genotype/ Allele frequencies	Cases n(%)	Controls n(%)	χ^2	Odds ratio(95%CI)	***p***-value
GG	30 (20)	45 (30)	0.29	1.18(0.75-1.62)	0.58
AA	84 (56)	41 (27.3)	14.1	3.07(1.24-2.27)	0.0002*
GA	36 (24)	64 (42.6)	21.7	0.27(0.15-0.47)	0.001*
G	96 (32)	154 (51.3)	0.17	1.10 (0.78-1.44)	0.67
A	204 (68)	146 (48.6)	23.07	2.24(1.26-1.81)	0.0001*
GG vs GA+AA (Dominant)	30/120	45/105	4.0	0.58(0.34-0.99)	0.06
AA vs GA+GG (Recessive)	84/66	41/109	25.3	3.38(2.08-5.48)	0.001*

Abbreviations: CI, confidence interval; OR, odds ratio.Calculated by chi-squared test.

Table 4. Association between serum 25(OH) D levels and FGTB risk.

Serum Vit D status	FGTB n=150	Control n=150	RR (95% CI)	OR (95% CI)	*p*-value
Insufficient vs deficient	14/124	50/28	0.26(0.16-0.42)	0.06(0.03-0.13)	0.001*
Insufficient vs Sufficient	14/12	50/72	1.53(0.76-3.08)	1.68(0.71-3.93)	0.229
Deficient vs Sufficient	124/12	28/72	5.71(3.36-9.69)	26.5(12.7-55.4)	0.001*

FGTB=Female Genital Tuberculosis, RR=risk ratio, OR=odds ratio, CI=confidence interval, 25(OH) D=25hydroxy vitamin D3.
Deficient: 25(OH) D< 10 ng/ml, Insufficient: 25(OH) D between 10-25 ng/ml, Sufficient: 25(OH) D>25ng/ml.

Table 5. Mean serum levels of 25(OH) D3 for each Cdx-2 genotypes.

Cdx-2	FGTB n=150 Mean±SD (ng/ml)	Control n=150 Mean±SD (ng/ml)	***p***value

GG	29.87±0.33	31.43±0.99	0.17
GA	19.33 ± 0.33	22.33 ± 1.33	0.09
AA	9.00 ± 0.47	28.80 ± 0.38	0.0001*

Figure 1. Correlation of conventional and molecular test.

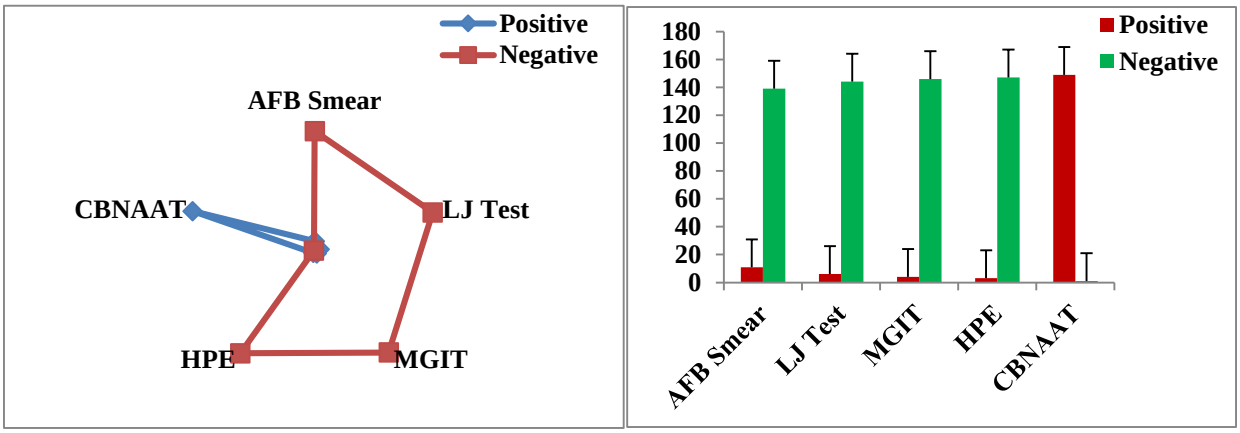


Figure 2. Genotypeandallele frequency distribution among FGTB case and controls.

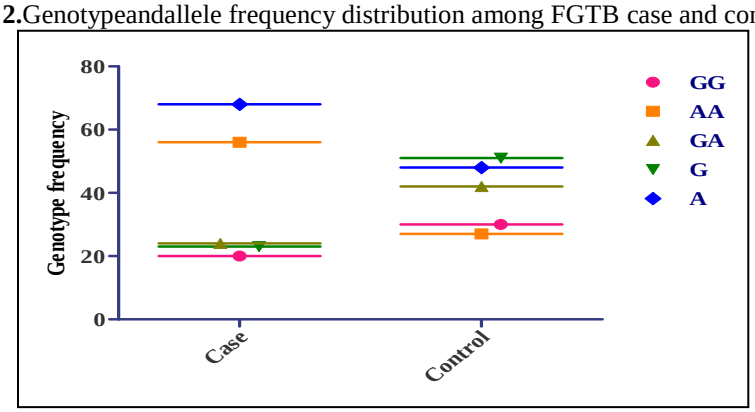
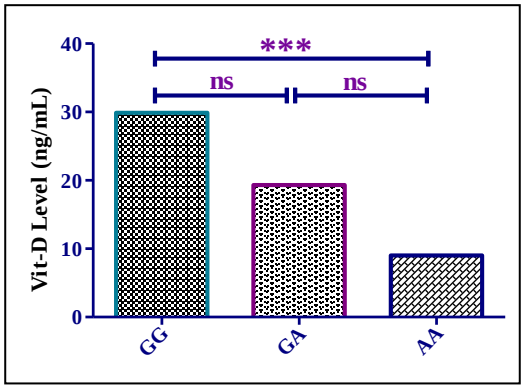


Figure 3. Mean ± SD vitamin D levels (ng/ml) as per genotypic distribution of Cdx-2 SNP.



Discussion

The findings of this study reveal that the involvement of VDR-Cdx2 gene polymorphism affecting serum 25(OH) D3 levels has a substantial connection to the etiology of GTB. Allelic variants in the VDR gene can result in significant receptor dysfunction, compromising vitamin D function and influencing immunological response. Furthermore, SNPs in the regulatory regions of VDR can affect its expression [23]. Many studies have found that a change in the transcriptional factor Cdx2 in the promoter region of the VDR binding site altered the transcriptional activity of the VDR gene in cancer, TB, and other disorders. The Cdx2 transcription factor A allele binds more effectively in breast

cancer, resulting in higher VDR transcriptional activity and lower breast cancer incidence, whereas the G allele has the reverse effect [24]. Similarly, in a case-control study conducted in Southern Pakistan, the GG genotype was linked to an increased risk of breast cancer [25].

We investigated VDR Cdx-2 polymorphism in response to female genital tuberculosis; in this regard, the genotypes AA (***p*** <0.0002*), GA (***p***<0.001*), and A allele (***p***< 0.0001*) were associated with an increased risk of developing FGTB. Patients with the "AA" genotype were connected to an increased risk of tuberculosis in the Indian population [27]. In a South Indian population, the promoter polymorphisms Cdx2 G/A and A1012G found that genotype "GG" of Cdx2 was related with protection and haplotype A-A (A allele of Cdx-2 and A allele of A1012G) with susceptibility to pulmonary tuberculosis [28].Furthermore, the role of VDR gene variations in immunological modulation was investigated. The Cdx2 "AA" and TaqI "tt" genotypes have been shown to influence chemokine-mediated inflammatory responses in T-cell subsets [29-30].There was a substantial association established between the Cdx-2 AA genotype and lower levels of MIP-1 and MIP-1. Reduced chemokine levels in the AA genotype at the infection site may result in poor *M.Tuberculisis*treatment and may be connected to an increased risk of TB [31], according to these investigations, which complement our findings.

The most important type of vitamin D in the body is 25-hydroxy vitamin D3, and its amount in the blood is a good indicator of how much vitamin D the body has. It exerts its effects via the vitamin D receptor (VDR), which is present in the majority of immune cells, including macrophages, B and T lymphocytes, neutrophils, and dendritic cells. In numerous studies, vitamin D deficiency has been linked to TB susceptibility [32-33]. A low level of serum vitamin 25(OH) D3 was shown to be substantially linked (***p***<0.0001*) with patients of genital TB in our investigation. The high prevalence of vitamin D deficiency among FGTB cases in the present study is consistent with a study by **Gautam S *et al***.who reported that vitamin D deficiency is strongly associated with the progression of active FGTB disease [34].According to **Kim JH *et al.***, a low serum 25(OH) D3 level may be a risk factor for active TB. **Meyer and Bornman *et al.***[35] shown that the VDR SNP (rs11568820, Cdx-2) has no effect on VDR regulation and is dependent on circulating vitamin D levels and VDR methylation. To validate this conclusion, we looked into the putative link between Cdx-2 SNP genotypes and serum vitamin D levels. The study finding of our investigation is the patients with genotype "GG" had 25(OH) D3 level (≥ 30 ng/ml), genotypes "GA" had25(OH) D3 level (< 20ng/ml), whereas genotype "AA" patients had deficient level (<10ng/ml, ***p***<0.0001) that was significantly associated with FGTB. This study finding could help avoid vitamin D deficiency by improving dietary behaviors and monitoring individuals with the risky "AA" genotype's circulating 25(OH) D3 levels. Ethnicity was found to be a very important factor in genetic function in pulmonary tuberculosis [36]. Different ethnic, geographical, and other factors all have an impact on gene polymorphism. Contradictory results have not supported this study, due to differences in genetic background among different races, which have also led to different genotype frequencies. Sample size, case and control groups of sources, inclusion criteria, and different technical factors may also lead to different results. The findings contribute to the growing body of evidence about the Cdx2 gene, which may be involved in the etiology of genital TB.

Limitation of the study

The limitation of this study was that there was no prior information available on the role of the Cdx2 gene polymorphism in FGTB disease progression. The present study prompts repetitive large-scale studies of the FGTB predisposition gene in larger sample sizes to reach statistical significance between the genotypes and the risk of FGTB, as well as to show interactions between the genes and the environment. Sequencing is needed for further analysis and to clearly elucidate the mechanisms by which the gene might affect disease progression.

Conclusion

The study concluded that SNPs in the VDR Cdx2 gene may influence serum 25(OH) D3 levels and one of the important contributors to an increased risk of GTB in patients. This research also implies that vitamin D deficiency may be predicted in people based on their genetic profile, and that these people should take vitamin D supplements on a daily basis. Consequently, to stop the evolution of vitamin D-dependent GTB, an evaluation of the VDR

genetic profile in addition to assessments of 25(OH) D3 concentrations may be beneficial.

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Authors'contributions

SPJ: Conceptualization, methodology, project administration, supervision.

US: Investigation, writing of the original draft, data curation, formal analysis.

AP: Writing, review and editing.

AJ: Data validation curation, software, resources.

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Declaration of interests

None

References

1- Global Tuberculosis Report. WHO (2022). https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report. Accessed October 27, 2022.

2- India TB Report (2022) https://tbcindia.gov.in/index1.php?lang=1&level=1&sublinkid=5613&lid=3658 Accessed March 24, 2022.

3- **Golden MP, Vikram HR**. Extrapulmonary tuberculosis: an overview. American family physician 2005;72(9):1761-8.

4- Jahromi BN, Parsanezhad ME, Ghane-Shirazi R. Female genital tuberculosis and infertility. International Journal of Gynecology & Obstetrics. 2001 Dec 1;75(3):269-72.

5- Haussler MR, Whitfield GK, Haussler CA, Hsieh JC, Thompson PD, Selznick SH, Dominguez CE, Jurutka PW. The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. Journal of bone and mineral research. 1998 Mar 1;13(3):325-49.

6- **Miyamoto KI, Kesterson RA, Yamamoto H, Taketani Y, Nishiwaki E, Tatsumi S**, Inoue Y, Morita K, Takeda E, Pike JW. Structural organization of the human vitamin D receptor chromosomal gene and its promoter. Molecular endocrinology. 1997 Jul 1;11(8):1165-79.DOI: 10.1210/mend.11.8.9951

7- **Kim JH, Park JS, Cho YJ, Yoon HI, Song JH, Lee CT**, Lee JH. Low serum 25-hydroxyvitamin D level: an independent risk factor for tuberculosis?. Clinical nutrition. 2014 Dec 1;33(6):1081-6.

8- **Panda S, Tiwari A, Luthra K, Sharma SK, Singh A**. Association of Fok1 VDR polymorphism with Vitamin D and its associated molecules in pulmonary tuberculosis patients and their household contacts. Scientific Reports. 2019 Oct 24;9(1):15251.DOI: 10.1038/s41598-019-51803-8

9- **Bellamy R, Ruwende C, Corrah T, McAdam KP, Thursz M, Whittle HC**, Hill AV. Tuberculosis and chronic hepatitis B virus infection in Africans and variation in the vitamin D receptor gene. The Journal of infectious diseases. 1999 Mar 1;179(3):721-4.. DOI: 10.1086/314614

10- **Joshi L, Ponnana M, Penmetsa SR, Nallari P, Valluri V, Gaddam S**. Serum Vitamin D Levels and VDR Polymorphisms (B sm I and F ok I) in Patients and their Household Contacts Susceptible to Tuberculosis. Scandinavian journal of immunology. 2014 Feb;79(2):113-9..DOI: 10.1111/sji.12127

11- **Selvaraj P, Narayanan PR, Reetha AM.** Association of vitamin D receptor genotypes with the susceptibility to pulmonary tuberculosis in female patients & resistance in female contracts. Indian Journal of Medical Research. 2000 May 1;111:172.PMID: 10943070

12- **Selvaraj P, Chandra G, Kurian SM, Reetha AM, Narayanan PR**. Association of vitamin D receptor gene variants of BsmI, ApaI and FokI polymorphisms with susceptibility or resistance to pulmonary tuberculosis. Current Science. 2003 Jun 25:1564-8.DOI: 10.1111/j.1744-313X.2008.0076x

13- **Selvaraj P, Chandra G, Jawahar MS, Rani MV, Rajeshwari DN, Narayanan PR.** 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. Journal of clinical immunology. 2004 Sep;24:523-32.DOI: 10.1023/B:JOCI.0000040923.07879.31

14- **Fang Y, Van Meurs JB, Bergink AP, Hofman A, Van Duijn CM, Van Leeuwen JP**, Pols HA, Uitterlinden AG. Cdx-2 polymorphism in the promoter region of the human vitamin D receptor gene determines susceptibility to fracture in the elderly. Journal of Bone and Mineral Research. 2003 Sep;18(9):1632-41.DOI: 10.1359/jbmr.2003.18.9.1632

15- **Arai H, Miyamoto KI, Yoshida M, Yamamoto H, Taketani Y, Morita K**, Kubota M, Yoshida S, Ikeda M, Watabe F, Kanemasa Y. The polymorphism in the caudal-related homeodomain protein Cdx-2 binding element in the human vitamin D receptor gene. Journal of Bone and Mineral Research. 2001 Jul;16(7):1256-64..DOI: 10.1359/jbmr.2001.16.7.1256

16- **Selvaraj P, Alagarasu K, Harishankar M, Vidyarani M, Narayanan PR.** Regulatory region polymorphisms of vitamin D receptor gene in pulmonary tuberculosis patients and normal healthy subjects of south India. International journal of immunogenetics. 2008 Jun;35(3):251-4..DOI: 10.1111/j.1744-313X.2008.00764.x

17- **Meyer V, Bornman L.** Cdx-2 polymorphism in the vitamin D receptor gene (VDR) marks VDR expression in monocyte/macrophages through VDR promoter methylation. Immunogenetics. 2018 Aug;70:523-32..DOI: 10.1007/s00251-018-1063-5

18- **Harishankar M, Selvaraj P.** Regulatory role of Cdx-2 and Taq I polymorphism of vitamin D receptor gene on chemokine expression in pulmonary tuberculosis. Human Immunology. 2016 Jun 1;77(6):498-505..DOI: 10.1016/j.humimm.2016.04.008

19- **Harishankar M, Selvaraj P.** Influence of Cdx2 and TaqI gene variants on vitamin D3 modulated intracellular chemokine positive T-cell subsets in pulmonary tuberculosis. Clinical therapeutics. 2017 May 1;39(5):946-57.DOI: 10.1016/j.clinthera.2017.04.003

20- **Gibney KB, MacGregor L, Leder K, Torresi J, Marshall C, Ebeling PR**, Biggs BA. Vitamin D deficiency is associated with tuberculosis and latent tuberculosis infection in immigrants from sub-Saharan Africa. Clinical infectious diseases. 2008 Feb 1;46(3):443-6..DOI: 10.1086/525268

21- **Nnoaham KE, Clarke A.** Low serum vitamin D levels and tuberculosis: a systematic review and meta-analysis. International journal of epidemiology. 2008 Feb 1;37(1):113-9.DOI: 10.1093/ije/dym247

22- **Kafle S, Basnet AK, Karki K, Magar MT, Shrestha S, Yadav RS.** Association of vitamin D deficiency with pulmonary tuberculosis: a systematic review and meta-analysis. Cureus. 2021 Sep 10;13(9).DOI: 10.7759/cureus.17883

23- de Albuquerque Borborema ME, de Souza Pereira JJ, dos Santos Peixoto A, Crovella S, Schindler HC, da Silva Rabello MC, de Azevêdo Silva J. Differential distribution in vitamin D receptor gene variants and expression profile in Northeast Brazil influences upon active pulmonary tuberculosis. Molecular Biology Reports. 2020 Sep;47:7317-22.

24- **Zhou ZC, Wang J, Cai ZH, Zhang QH, Cai ZX, Wu JH.** Association between vitamin D receptor gene Cdx2 polymorphism and breast cancer susceptibility. Tumor Biology. 2013 Dec;34:3437-41.DOI: 10.1007/s13277-013-0919-4

25- **Iqbal MU, Khan TA, Maqbool SA.** Vitamin D receptor Cdx-2 polymorphism and premenopausal breast cancer risk in southern Pakistani patients. PLoS One. 2015 Mar 23;10(3):e0122657.DOI: 10.1371/journal.pone.0122657.

26- **Rehman M, Mahboob T, Shahid SM.** Possible association of Vitamin D receptor, caudal-related homeobox 2 polymorphism with the risk of cancer. International Journal of Health Sciences. 2021 Mar;15(2):9.PMID: 33708039

27- Alagarasu K, Selvaraj P, Swaminathan S, Narendran G, Narayanan PR. 5′ regulatory and 3′ untranslated region polymorphisms of vitamin D receptor gene in South Indian HIV and HIV–TB patients. Journal of clinical immunology. 2009 Mar;29:196-204.

28- **Selvaraj P, Alagarasu K, Harishankar M, Vidyarani M, Narayanan PR.** Regulatory region polymorphisms of vitamin D receptor gene in pulmonary tuberculosis patients and normal healthy subjects of south India. International journal of immunogenetics. 2008 Jun;35(3):251-4

29- **Harishankar M, Selvaraj P.** Regulatory role of Cdx-2 and Taq I polymorphism of vitamin D receptor gene on chemokine expression in pulmonary tuberculosis. Human Immunology. 2016 Jun 1;77(6):498-505.

30- **Harishankar M, Selvaraj P.** Influence of Cdx2 and TaqI gene variants on vitamin D3 modulated intracellular chemokine positive T-cell subsets in pulmonary tuberculosis. Clinical therapeutics. 2017 May 1;39(5):946-57.

31- **Harishankar M, Selvaraj P.** Regulatory role of Cdx-2 and Taq I polymorphism of vitamin D receptor gene on chemokine expression in pulmonary tuberculosis. Human Immunology. 2016 Jun 1;77(6):498-505.

32- Dana PM, Sadoughi F, Hallajzadeh J, Asemi Z, Mansournia MA, Yousefi B, Momen-Heravi M. An insight into the sex differences in COVID-19 patients: what are the possible causes?.Prehospital and disaster medicine. 2020 Aug;35(4):438-41.

33- **Martineau AR, Leandro AC, Anderson ST, Newton SM, Wilkinson KA, Nicol MP,**Pienaar SM, Skolimowska KH, Rocha MA, Rolla VC, Levin M. Association between Gc genotype and susceptibility to TB is dependent on vitamin D status. European Respiratory Journal. 2010 May 1;35(5):1106-12.

34- **Gautam S, Jain A, Akhtar S, Priyadarshini A, Jaiswar SP.** Serum vitamin D level as a risk factor for female Genital tuberculosis (FGTB).

Journal of Clinical and Diagnostic Research: JCDR. 2017 Sep;11(9):DC18

35- Meyer V, Bornman L. Cdx-2 polymorphism in the vitamin D receptor gene (VDR) marks VDR expression in monocyte/macrophages through VDR promoter methylation. Immunogenetics. 2018 Aug;70:523-32.

36- **Sinaga BY, Amin M, Siregar Y, Sarumpaet SM.** Correlation between Vitamin D receptor gene FOKI and BSMI polymorphisms and the susceptibility to pulmonary tuberculosis in an Indonesian Batak-ethnic population. ActaMedicaIndonesiana. 2014 Oct 1;46(4).PMID: 25633543

SiddiquiU, PriyadarshiniA, JaiswarS P, JainA. Linking 5'-promoter region of Cdx2 VDR polymorphism and serum vitamin D association in female genital tuberculosis.Microbes Infect Dis 2024; Article-In-Press, **DOI:** 10.21608/mid.2024.250347.1672.