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Expression of *Vitamin D Receptor* (*VDR*) gene and *VDR* polymorphism rs11574113 in pulmonary tuberculosis patients and their household contacts

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ABSTRACT

The vitamin D receptor mediates the effect of vitamin D, which has received substantial attention in its involvement in reducing the risk of tuberculosis (TB). This study sought to see if *VDR* gene expression and *VDR* SNP rs11574113 was linked to a person's likelihood of developing tuberculosis. New Pulmonary Tuberculosis (PTB) *VDR* gene expression and Household Contact (HC) samples *VDR* gene expressions were examined by the quantitative real-time PCR (qPCR) method, while *VDR* SNP rs11574113 was identified by PCR followed by sequencing. Latent TB infection in household contacts was screened using an Interferon-Gamma Release Assay (IGRA). Eighty-three PTB, 77 HCs (45 positive-IGRA and 28 negative-IGRA) were involved in this study. Among the 45 positive-IGRA HC, 28 (62.2%) were spouses of index cases. IGRA results showed that 28 out of 37 (75.7%) samples of all spouses recruited have positive IGRA results. *VDR* gene expression of PTB was 0.2 and 0.25 downregulated compared to negative-IGRA and positive-IGRA HC, respectively ($p = 0.014$ and $p = 0.01$). *VDR* gene expression of positive-IGRA was also 0.788 downregulated compared to negative-IGRA HC but did not reach statistical significance ($p = 0.945$). The high expression of the *VDR* gene may be influenced by variant of *VDR* SNP rs11574113. G/G genotype and G/C genotype were associated with higher VDR gene expression compared to C/C genotype ($OR^a = 9.754$ 95% CI 1.482–64.195, $p = 0.018$ and $OR^a = 9.723$ 95% CI 1.416–66.751, $p = 0.021$, respectively). However, rs11574113 was not found to be associated with TB incidence and IGRA positivity. Approximately 20% of the people with PTB have higher levels of *VDR* gene expression than those who were not ($OR^a = 0.736$ 95% CI 0.542–0.998, $p = 0.049$). *VDR* gene expression may be associated with the susceptibility of tuberculosis. This study also shows an immense rate of TB infection among spouses of PTB patients based on IGRA.

Abbreviations: Mtb, *Mycobacterium tuberculosis*; *VDR*, *Vitamin D Receptor*; TB, tuberculosis; PTB, pulmonary tuberculosis; IGRA, Interferon-Gamma Release Assay; HC, household contact; *GAPDH*, glyceraldehyde-3 phosphate dehydrogenase; qPCR, quantitative real-time PCR; Ct, cycle threshold; TNF, tumor necrosis factor; CYP27B1, *cytochrome p450 27B1*; UTR, untranslated region; SNP, single nucleotide polymorphism.

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1. Introduction

With about 10 million infections and 1.4 million deaths, tuberculosis is a major global public health concern. Indonesia is among the eight countries that contribute to two-thirds of TB cases worldwide and ranks second in the highest TB incidence (8.5%), with case notifications increasing by about 69% from 2015 to 2019 (World Health Organization, 2020). TB elimination can be achieved not only by effective active TB therapy but also by early case detection to stop transmission through various strategies for preventing new infections and neutralizing the existing dormant infections. Without tackling reservoirs of latent infection, the WHO's "End TB" strategy is difficult to be accomplished (Dye et al., 2013). Around one-fourth of the population worldwide has been infected by *Mycobacterium tuberculosis* (Mtb), the tuberculosis-provoking bacteria. Around 5–10% of these latent cases show symptoms of active TB (Sterling et al., 2020).

In TB contact investigations, household contacts are most likely infected by people with active TB (index case) (Lee, 2016; Fox et al., 2013). Several studies of tuberculosis household contact showed that some contacts remained negative based on TB infection tests, even after prolonged exposure (Fox et al., 2013; Reichler et al., 2018). These observations raise the question of whether some people might be able to withstand the *Mtb* infection or quickly clear the infection. Learning more about who these people are and what immunological or genetic factors allow them to fight infection may help develop better biomedical prevention and treatment tools, including a TB vaccine.

Some studies have suggested a link between the genetic make-up of the host and the prevalence of tuberculosis (Qidwai et al., 2012; van Tong et al., 2017), one of which is the *Vitamin D Receptor* (*VDR*) gene (Xu and Shen, 2019; Cao et al., 2016; Huang et al., 2015). The *VDR* gene is of great interest since it mediates the effects of vitamin D, which has been known to modulate the activity of monocytes-macrophages in the body, which are important in regulating innate defense against infectious pathogens such as *Mtb* (Henrique et al., 2017; Ashenafi et al., 2018). In numerous studies, vitamin D shortage is associated with TB susceptibility (Tessema et al., 2017; Huang et al., 2017; Junaid et al., 2016). However, some reports did not find a significant discrepancy in vitamin D quantity in TB and non-TB individuals (Ashenafi et al., 2018; Koo et al., 2012; Yuvaraj et al., 2016; Sarin et al., 2016). In addition, randomized controlled trials have not found a significant effect on vitamin D3 supplementation to increase the rate of acid-fast bacilli conversion in TB patients (Ganmaa et al., 2017; Tukvadze et al., 2015; Bekele et al., 2018) or reduce the risk of TB infection (Ganmaa et al., 2020). Intervention with vitamin D3 appears to be modified by genetic variants in *VDR* (Ganmaa et al., 2017). Consequently, the action of vitamin D may be contingent on the genotype and activity of the *VDR* (Sutaria et al., 2014).

The binding of VDR with calcitriol, vitamin D active form, induces the synthesis of cathelicidin (LL37). LL37 plays an essential role in mycobactericidal activity (Coussens et al., 2015; Belyaeva et al., 2017; Sonawane et al., 2011) and is involved in the autophagy process by enhancing the fusion of mycobacterial phagosomes with lysosomes (Chung et al., 2020; Choi et al., 2012; Paik et al., 2019). The binding of the calcitriol to the intracellular VDR also modulates approximately 900 genes involved in several physiological processes in both natural and adaptive immunity (Bizzaro et al., 2017). VDR helps to regulate the adaptive immune system by preventing over-response by suppressing lymphocyte proliferation and inhibiting the generation of proinflammatory cytokines. (Sutaria et al., 2014). The researchers identified genetic variants of the *VDR* gene as being linked to a risk of TB based on the idea that genetic variations may cause anomalies and influence the immunological function of the *VDR* gene (Wilkinson et al., 2013).

Research so far has been investigating the association between *VDR* polymorphisms with the development of active TB with varied results (Junaid et al., 2016; Medapati et al., 2017; Salimi et al., 2015; Mahmoud and Ali, 2014; Silva-Ramírez et al., 2019). There is still a lack of studies about *VDR* gene expression in those infected with *Mtb*. The purpose of this study was to investigate the association between gene expression of *VDR* and vulnerability to TB. In this study, we analyze the gene expression of *VDR* in TB patients compared to controls who were known to be exposed to the *Mtb* bacilli, in this case, household contacts (HC). This study also aimed to compare *VDR* gene expression between positive and negative IGRA (Interferon-Gamma Release Assay) HC. IGRA was performed on household contacts to determine the response of cellular immunity (T cells) to TB infection (Trajman et al., 2013; Eom et al., 2018). In addition, the 3'UTR (untranslated region) VDR polymorphism, rs11574113, was also identified to assess its association with *VDR* gene expression as well as susceptibility to TB. The SNP (single nucleotide polymorphism) rs11574113 G > C is located at intron 8 and has been investigated in only a few studies regarding its association with tuberculosis (Andraos, 2011; Hu et al., 2016).

2. Material and methods

2.1. Research design and sample recruitment

The study design was cross-sectional, involving PTB patients and their HC as participants. We recruited the PTB patients from The Community Lung Health Center or Balai Besar Kesehatan Paru Masyarakat (BBKPM) Makassar, South Sulawesi, Indonesia, which is one of the main TB referral health center in South Sulawesi Province. PTB patients included in this study were pulmonary TB patients aged more than 15 years old that diagnosed with active TB for the first time based on clinical manifestations, chest X-ray, and positive microscopic smears, which were further confirmed through TB culture. The exclusion criteria were HIV-positive patients which were examined by using SD Bioline. In this study, the household contacts were contacts aged 15 and above who resided for at least six months in the same residence as TB patients (Halliday et al., 2017), had no clinical symptoms of TB, and never had anti-tuberculosis drug therapy. All participants have signed the consent form.

We collected blood samples from PTB and HC. Before RNA extraction, the blood samples were kept at −80 °C. In order to test for latent TB infection in HC, QuantiFERON Gold Plus TB Test (Qiagen, Germany) was utilized, according to the manufacturer guide (Qiagen, 2016). The positive sputum samples of PTB patients were decontaminated and the culture process proceeded as a gold standard of TB diagnosis in the Hasanuddin University Medical Research Center Laboratory in Makassar, Indonesia.

This research has been approved by The Committee of Research Ethics, Faculty of Medicine, Universitas Hasanuddin, Makassar, South Sulawesi, Indonesia (No. 583/ H4.8.4.5.31/ PP36-KOMETIK/ 2018). All study participants gave their written consent.

2.2. VDR gene expression by real-time PCR

2.2.1. RNA extraction and complementary DNA synthesis

Total RNA was extracted from whole blood samples, using Tiangen RNAprep Pure Blood Kit for purification of total RNA from human whole blood Cat. No. 4992238 (Tiangen, Biotech, Beijing, China). Concentrated RNA was rinsed with 50 μl RNAse free H_2O and utilized to complementary DNA (cDNA).

cDNA has been synthesized by using the iScript™ cDNA Synthesis Kit Master Cat. No. 178890 (Bio-Rad, California, USA). The reaction mixture consisted of 5 μg of the total RNA, 1 μl iScript Reverse Transcriptase, 4 μl 5× the iScript reaction mixture, and nuclease-free water up to 25 μl. The reaction mixture was subsequently incubated in a thermal cycler (Bio-Rad, USA), with 5-minute priming at 25 °C, 20 min of reverse transcription (RT), and 95 °C for a minute of RT inactivation. Until utilized for real-time PCR, cDNA was kept at −20 °C.

2.2.2. VDR gene expression by real-time quantitative polymerase chain reaction

The determination of the strength of gene expression (upregulation or downregulation) of *VDR* used SsoFastTM EvaGreen® Supermix Cat. No. 172–5200 (Bio-Rad, California, USA), and *glyceraldehyde-3 phosphate dehydrogenase* (*GAPDH*) as the housekeeping gene with quantitative real-time PCR (qPCR) method. Prior to amplification, the master mix was prepared by mixing 5 μl of SsoFastTM EvaGreen® Supermix, 1 μl of forward primer, 1 μl of reverse primer (each 10 nM primary concentration), the volume of cDNA corresponds to the cDNA concentration of 150 ng/μl, and the volume of nuclease-free water adjusted to reach 3 μl of the cDNA and nuclease-free water mixture. The primers used in this study were F: 5′-CGC ATC ATT GCC ATA CTG CTG G-3′ and R: 5′-CCA CCA TCA TTC ACA CGA ACT GG-3′ (Salehi-tabar et al., 2018) to amplify the *VDR* gene (NCBI (The National Center for Biotechnology Information) Reference sequence: NM_000376) and F: 5′-CCT GCA CCA CCA ACT GCC TTA-3′ and R: 5′-GGC CAT CCA CAG TCT TCT GAG-3′ to amplify *GAPDH* gene. The real-time PCR engine used was the CFX96 Touch Real-time PCR Detection System (Bio-Rad, California, USA) which is connected to the CFX Manager ™ Software # 1845000 for Windows, with qPCR cycles: initial denaturation at 95 °C for 30 s, followed by 45 cycles of denaturation at 95 °C for 5 s, annealing at 57 °C for 30 s, and plate reading. Melt-curve was generated by increasing the temperature from 65.0 to 95.0 °C with increment 0.5 °C/5 s, followed by plate reading.

All analyses were performed by a real-time PCR method using Bio-Rad CFX™ Manager Software (version 3.1, Bio-Rad Lab. Inc., CA, USA). The level of gene expression was determined by the appearance of the amplicon signal on the graph. The signal detected in the small number of cycles indicates a higher level of gene expression. After the amplification process, a dissociation curve was obtained, which then analyzed for its relative expression using a method that compares the Ct (cycle threshold) value of the *VDR* gene ($Ct_{target\ genes}$) with the selected reference value, i.e. the expression level of the *GAPDH* (Ct_{ref}), by calculating:

$$\Delta Ct = Ct_{target\ genes} - Ct_{ref}.$$

Remarks:

ΔCt: The distinction between the *VDR* Ct and the *GAPDH* Ct value.

$Ct_{target\ genes}$: Ct value of *VDR* gene.

Ct_{ref}: Ct value of *GAPDH* gene.

Then a comparison was obtained with the formula of the gene expression:

$$\text{Relative VDR gene expression} = 2^{(-\Delta\Delta Ct)}.$$

Remarks:

Relative *VDR* gene expression: *VDR* gene expression of the target to control group ratio.

ΔΔCt: The difference between ΔCt of control and target group (Livak and Schmittgen, 2001).

2.3. VDR polymorphism (SNP rs11574113)

2.3.1. DNA extraction

A total of 200 μl of blood samples (plasma and buffy coat) were put into a sterile 1.5 ml microcentrifuge tube. 20 μl of Proteinase K was added and subsequently homogenized by pipetting, then incubated at 60° C for 5 min. DNA extraction was performed using the gSYNC™ DNA Extraction Kit (Geneaid, Taiwan) according to the kit protocol. Extraction results were stored at -80° C until used as a PCR template.

2.3.2. DNA amplification and sequencing

Primers used to amplify a 500 bp of the 3′ UTR of *VDR* (*SNP* rs11574113) were F 5′-CAG AGC ATG GAC AGG GAG CAA-3′ and R 5′-ACT TCG AGC ACA AGG GGC GTT AG-3′. The PCR reaction mixture consisted of 20 μl of the Taq Polymerase Kapa Biosystem Enzyme (Roche, USA), 1 μl of forward and reverse primers with a concentration of 10 M, 7–10 μl DNA samples, and nuclease-free water until the total reaction mixture reached 50 μl. Amplification was carried out with a thermal cycler machine (Bio-Rad, USA) with the PCR condition: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, extension at 72 °C for a minute, and final extension at 72 °C for 5 min. The PCR product was then electrophoresed on GelRed® (Biotium, Inc., Fremont, U.S)-stained 2% Agarose gel with an electrophoresis machine (Bio-Rad, USA) then visualized under UV light using the Gel Doc™ XR machine (Bio-Rad, USA) and Quantity One software version 4.5 (Bio-Rad, USA).

The PCR product was then sent to the 1st Base Laboratory, Malaysia for direct sequencing (Sanger Sequencing). The sequencing results obtained were subsequently analyzed using Bioedit for Windows software version 7.2 (Tom Hall) with NCBI RefSequence: NG_008731.1 (*Homo sapiens* vitamin D receptor (VDR), RefSeqGene on chromosome 12) as reference gene.

2.4. Statistical analysis

Statistical analyses were performed using the IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp. Armonk, NY, USA). The chi-square test was used to determine differences in nominal data between groups. To compare parametric data between two groups and three groups, *t*-test and One-way ANOVA were used, respectively. Meanwhile, the Mann–Whitney test and Kruskal–Wallis test were used to compare non-parametric data between three groups, respectively. qPCR data were evaluated in real-time with the software Bio-Rad CFX™ Manager (version 3.1, Bio-Rad Lab. Inc., Hercules, CA, USA). Visualization of sequencing chromatogram and alignment to reference sequence (*Homo sapiens vitamin D receptor* (*VDR*), RefSeqGene on chromosome 12 NCBI RefSequence: NG_008731.1:g.64915G > C) was performed by using Bioedit 7.2.5 (Alzohairy, 2011; Hall, 1999). SNPStats web-based software (https://www.snpstats.net) was used to analyze the Hardy-Weinberg equilibrium and association with active TB and positivity of IGRA, which was performed using a logistic regression model corrected for age, sex, and smoking status (Solé et al., 2006). A generalized linear model was used to examine the impact of factors to contribute to the incidence of PTB and IGRA positivity. Besides, it was also used to estimate the relationship between *VDR* polymorphism (*SNP* rs11574113) and *VDR* gene expression. In all studies, p-value ≤ 0.05 was statistically significant.

3. Result

3.1. Participants characterization

The characterization of the study participants is described in Table 1. In this study, positive IGRA was found in 61.64% (45/73) of household contacts. The age category across the groups did not differ ($p = 0.281$). Male was significantly dominant in TB group (53%) than in HC group, both positive (31.1%) and negative (17.9%) IGRA ($p = 0.001$). However, there was no substantial difference in gender between positive and negative IGRA HC ($p = 0.21$). In addition, the acid fast-bacilli of index cases was not significantly different in positive IGRA HC compared to negative IGRA HC. There were significant differences in BMI between all three groups ($p < 0.0001$), as well as between positive and negative IGRA HC ($p = 0.0018$). Underweight participants were more common in the TB group (48.1%), and obese participants were more in the negative IGRA HC group (59.3%). Spouses were found more in positive IGRA HC than other relationships (62.2%).

Table 1
Samples characterization.

	PTB $N = 83$	(+) IGRA HC $N = 45$	(−) IGRA HC $N = 28$	p-Value†	p-Value‡
Gender				**0.001***	0.21
• Male	44 (53%)	14 (31.1%)	5 (17.9%)		
• Female	39 (47%)	31 (68.9%)	23 (82.1%)		
Age (years)				0.281	0.325
• 15–39	36 (43.4%)	22 (48.9%)	17 (60.7%)		
• ≥40	47 (56.6%)	23 (51.1%)	11 (39.3%)		
BMI classification (kg/m^2)				**<0.0001***	**0.018***
• Underweight (<18.5)	39 (48.1%)	6 (13.3%)	2 (7.4%)		
• Normal weight (18.5–22.9)	35 (43.2%)	19 (42.2%)	6 (22.2%)		
• Overweight (23–24.9)	3 (3.7%)	10 (22.2%)	3 (11.1%)		
• Obese (≥25)	4 (4.9%)		16 (59.3%)		
Smoker	43 (51.8%)	11 (24.4%)	4 (14.3%)	**<0.0001***	0.296
Smear microscopic (PTB only)	37 (44.6%)				
• AFB 1+	35 (42.2%)				
• AFB 2+	11 (13.3%)				
• AFB 3+					
Smear microscopic of the index case (HC only)		25 (55.6%)	13 (46.4%)		0.46
• AFB 1+		13 (28.9%)	12 (42.9%)		
• AFB 2+		7 (15.6%)	3 (10.7%)		
• AFB 3+					
Relationship to index cases (HC only)		11 (24.4%)	9 (32.1%)		**0.025***
• Parent/son/daughter		28 (62.2%)	9 (32.1%)		
• Spouse		6 (13.3%)	10 (35.7%)		
• Others					

Remarks: † Chi-square test (among three groups); ‡ Chi-square test between (+) IGRA HC and (−) IGRA HC; * significant p-value: $p < 0.05$ (bold).
PTB: new-active pulmonary tuberculosis patient; HC: household contact; (+) IGRA: positive IGRA; (−) IGRA: negative IGRA; BMI: body mass index.

3.2. VDR gene expression in pulmonary tuberculosis patients and household contacts

Based on the qPCR results (Table 2), *VDR* gene expression in negative IGRA HC was around five times higher than in PTB ($p = 0.014$), and *VDR* gene expression in positive IGRA HC was approximately four times higher than in PTB ($p = 0.011$). These results indicated that *VDR* gene expression of PTB was 0.2 (1:5) and 0.25 (1:4) downregulated compared to negative IGRA and positive IGRA HC, respectively. *VDR* gene expression in negative IGRA HC was higher than those in positive IGRA HC, but not significant ($p = 0.945$).

3.3. VDR SNP rs11574113 analysis

The sequencing results of the *VDR SNP rs11574113* are shown in Supplementary files: Fig. 1. The results obtained conform to the Hardy-Weinberg equilibrium. The G/G genotype dominated the samples in this study, both in the PTB group (66/83 [79.5%]) and household contacts (59/73 [80.8%]). Meanwhile, the proportion of genotype C/C was the least in this study and was detected in only two samples of participants. In addition, it was found in the PTB group only (2/83 [2.4%]), thus the value of OR of this genotype was unable to determine. The distribution of the genotype of the *VDR* SNP rs11574113 and its relationship to the incidence of active pulmonary tuberculosis and IGRA positivity are shown in Table 3 and Table 4.

Table 2
Comparison of relative quantitative *VDR* gene expression between active pulmonary TB, positive IGRA, and negative IGRA household contacts.

Biological Group	Expression (95% CI)	p-Value	p-Value ANOVA†
(−) IGRA HC	**5.067 (1.149–22.339)**[a]	**0.014***	**0.013***
(+) IGRA HC	**3.994 (1.443–11.051)**[a]	**0.011***	
PTB	1	–	
(−) IGRA HC / (+) IGRA HC	1.269 (0.219–7.339)[b]	0.945	

Remarks: † the significance between three groups. * significant p-value: $p < 0.05$ (bold).
PTB: new-active pulmonary tuberculosis patient; HC: household contact; (+) IGRA: positive IGRA; (−) IGRA: negative IGRA.
[a] PTB as reference.
[b] (+) IGRA HC as the reference.

The *VDR* gene expression of each genotype can be seen in Fig. 1. The *VDR* gene expression of the G/G genotype group was not significantly different from that of G/C ($p = 0.782$). However, when compared with the C/C genotype group, the expression of the *VDR* gene was significantly higher in the G/G and G/C genotype groups ($p = 0.022$ and $p = 0.03$, respectively). By using generalized linear model analysis, G/G genotype and G/C genotype were associated with higher *VDR* gene expression compared to C/C genotype (OR[a] = 9.754 95% CI 1.482–64.195, $p = 0.018$ and OR[a] = 9.723 95% CI 1.416–66.751, $p = 0.021$, respectively). The differences in the expression of the *VDR* gene by clustering the genotype of *VDR* SNP rs11574113 in each sample group are depicted in Fig. 2. In the PTB group, the *VDR* gene expression was lower in the C/C genotype group ($p = 0.037$), while in the IGRA negative HC group, the *VDR* gene expression was significantly higher in the G/C group ($p = 0.042$).

3.4. Association of VDR gene expression with active pulmonary tuberculosis and IGRA positivity

The *VDR* genes expression was found to be associated with active pulmonary tuberculosis after adjustments to other variables (OR[a] =

Table 3
Genotype distribution of *VDR* SNP rs11574113 and its association to active pulmonary tuberculosis.

Model	Genotype	PTB $N = 83$	Household contact $N = 73$	OR 95% CI	p-Value
Codominant	G/G	66 (79.5%)	59 (80.8%)	1	0.2
	G/C	15 (18.1%)	14 (19.2%)	0.76 (0.29–2.02)	
	C/C	2 (2.4%)	0 (0%)	NA (0.00-NA)	
Dominant	G/G	66 (79.5%)	59 (80.8%)	1	0.81
	G/C-C/C	17 (20.5%)	14 (19.2%)	0.89 (0.34–2.31)	
Recessive	G/G-G/C	81 (97.6%)	73 (100%)	1	0.09
	C/C	2 (2.4%)	0 (0%)	NA (0.00-NA)	

Remarks: PTB: new-active pulmonary tuberculosis patient. OR: odds ratio.

Table 4
Genotype distribution of *VDR* SNP rs11574113 and its association to active pulmonary tuberculosis.

Model	Genotype	(+) IGRA HC $N = 45$	(−) IGRA HC $N = 28$	OR 95% CI	*p*-Value
–	G/G	35 (77.8%)	24 (85.7%)	1	0.53
	G/C	10 (22.2%)	4 (14.3%)	1.56 (0.39–6.19)	

Remarks: HC household contact; (+) IGRA: positive IGRA; (−) IGRA: negative IGRA; OR: odds ratio.

0.736 95% CI 0.542–0.998, $p = 0.049$), as shown in Table 5. However, the association between *VDR* gene expression and IGRA positivity was not found significant in this study (OR[a] = 0.783 95% CI 0.539–1.137, $p = 0.198$), as shown in Table 6.

4. Discussion

Early case detection and prevention of new infections are crucial in TB eradication efforts. Household contacts of tuberculosis patients are one group that has a high risk of suffering from tuberculosis (Fox et al., 2013). Household contact tracing of tuberculosis patients proved to be a better tool in detecting new tuberculosis cases than passive-case finding alone (Fox et al., 2018). Previous studies have revealed that some of these household contacts who had been exposed to *Mtb* for a long time did not experience infection, and the tuberculosis infection test results were still negative (Fox et al., 2013; Reichler et al., 2018). Several factors including genetic and immunological factors were investigated concerning an individual's resistance to tuberculosis infection (Allen et al., 2015; BoseDasgupta and Pieters, 2014; Wu et al., 2019; Harishankar et al., 2018).

In this study, *VDR* gene expression of PTB patients was significantly lower when compared to positive and negative IGRA HC, as shown in Fig. 1. This finding was similar to Panda's study, which also demonstrated the lower *VDR* expression in the active TB patient group than in household contacts with a negative tuberculin skin test. This result was presumably due to *Mtb*'s efforts to decrease *VDR* gene expression for its benefit, to prevent the host's immune system and finally remain in the host (Mangin et al., 2014). Supporting this hypothesis, a study by Padhi et al. demonstrated that one of the *Mtb* lipoproteins, LprE, may inhibit CYP27B1 and VDR molecules to support intracellular *Mtb* survival (Padhi et al., 2019). When bacterial ligands downregulate *VDR*, the receptor is unable to express the enzymes essential to keep calcitriol in the normal range, and high calcitriol levels may occur (Miao and Goltzman, 2021). Increased calcitriol lowers VDR competency, suppresses macrophage activity, and may block the nuclear factor kappa-B pathway (Mangin et al., 2014).

In line with the results of this study, a previous microarray-based study demonstrated that *VDR* expression in *Mtb*-stimulated macrophages (U937) was downregulated (Xu et al., 2003). Contrary, a subsequent study that examined *VDR* mRNA expression in macrophages of TB patients stimulated with *Mtb*, showed an increase in *VDR* mRNA expression (Selvaraj et al., 2009; Fiske et al., 2019). This contrasting result may be due to the differences in the macrophage model used. Xu et al. used the U937 macrophage model (cell line) (Xu et al., 2003), while Selvaraj et al. and Fiske et al. used macrophages from TB patients, which demonstrated "trained" immunity from previous *Mtb* exposure, whereas macrophages from these patients remained "naïve" and responded quickly after re-stimulation with *Mtb* (Selvaraj et al., 2009; Fiske et al., 2019).

In addition to the efforts of *Mtb* itself, the low expression of the *VDR* gene experienced by TB patients themselves may lead to disruption of their immunity to be susceptible to tuberculosis. A decrease in the mRNA level of the *VDR* gene in TB cases indicates a disturbance in the activation process of inflammatory cytokines and innate immune cells, thereby reducing immunity to TB disease (Maruthai et al., 2020). Thus, the low expression of the *VDR* gene in TB patients may be a risk factor for TB infection or as a result of *Mtb* infection, as some experts believe (Mangin et al., 2014).

The expression of the *VDR* gene for positive IGRA HC was found to be lower than that of negative IGRA HC but did not reach statistical significance (Table 3). To the best of our knowledge, this was the first study conducted to investigate the difference of the *VDR* expression gene

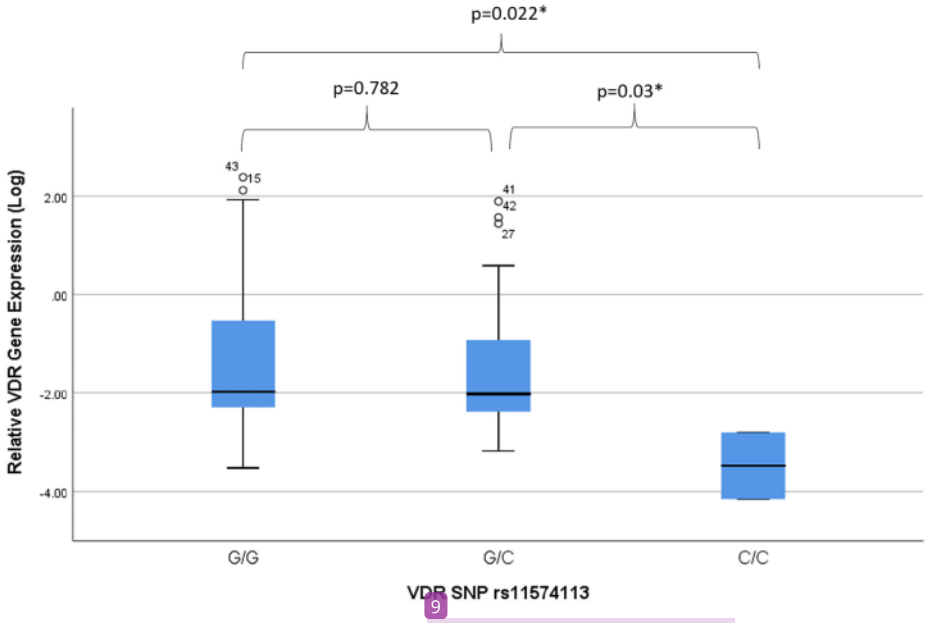

Fig. 1. *VDR* gene expression in each genotype of *VDR* SNP rs1154713. Sign* indicates a significant difference ($p < 0.05$) of *VDR* gene expression between two groups calculated by the Mann–Whitney test.

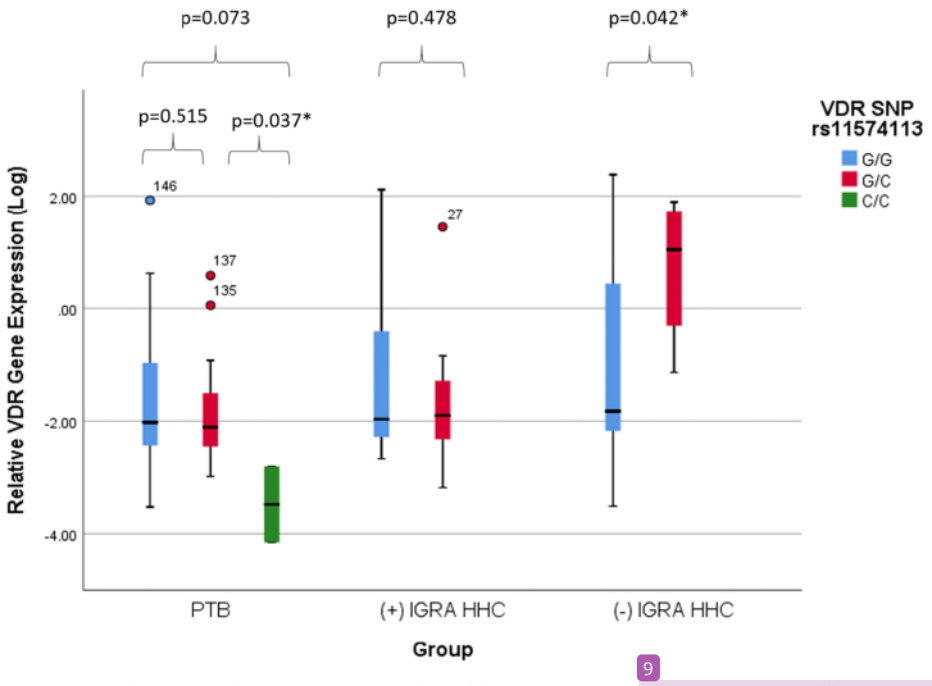

Fig. 2. *VDR* gene expression in each genotype of *VDR* SNP rs1154713 (clustered by the group). Sign* indicates a significant difference ($p < 0.05$) of *VDR* gene expression between two groups and three groups calculated by Mann–Whitney and Kruskal–Wallis test.

Table 5
Association of variables and active pulmonary tuberculosis in multivariate analysis.

Variable	OR[a] (95% CI)	*p*-Value[a]
Age	1.022 (0.993–1.051)	0.142
Gender		
Male	0.97 (0.342–2.749)	0.954
Female	1	
BMI	**0.745 (0.664–0.837)**	**<0.0001***
Smoker		
No	**0.323 (0.112–0.933)**	**0.037***
Yes	1	
Relative *VDR* gene expression (Log)	**0.736 (0.542–0.998)**	**0.049***
VDR SNP rs11574113		
G/G	1.155 (0.437–3.051)	0.772
G/C-C/C	1	

Remarks: BMI: body mass index; *VDR*: *Vitamin D Receptor*; OR: odds ratio.
* Significant *p*-value : $p \leq 0.05$ (bold).
[a] Analysis was adjusted to age, sex, BMI, smoking history, relative *VDR* gene expression (Log) and *VDR* SNP rs11574113 by using household contacts as a reference category.

Table 6
Association of variables and IGRA positivity of household contacts in multivariate analysis.

Variable	OR[a] (95% CI)	*p*-Value[a]
Age	1.001 (0.955–1.049)	0.97
Gender		
Male	1.336 (0.272–6.571)	0.772
Female	1	
BMI	**0.831 (0.733–0.942)**	**0.004***
Smoker		
No	0.466 (0.084–3.106)	0.466
Yes	1	
Smear microscopic of the index case		
AFB 1+	0.538 (0.087–3.348)	0.507
AFB 2+	0.399 (0.061–2.593)	0.336
AFB 3+	1	
Relation to the index case		
Parent/son/daughter	2.157 (0.451–10.322)	0.336
Spouse	**9.201 (1.757–48.192)**	**0.009***
Others	1	
Relative *VDR* gene expression (Log)	0.783 (0.539–1.137)	0.198
VDR SNP rs11574113		
G/G	0.564 (0.122–2.62)	**0.465**
G/C-C/C	1	

Remarks: BMI: Body mass index; AFB: acid-fast bacilli; *VDR*: *Vitamin D Receptor*; OR: odds ratio.
* Significant *p*-value : $p < 0.05$ (bold).
[a] Analysis was adjusted to age, sex, BMI, smoking history, AFB of the index case, relation to the index case, relative *VDR* gene expression (Log) and *VDR* SNP rs11574113 by using negative IGRA household contacts as a reference category.

between positive and negative IGRA people. IGRA-positive results in household contacts indicated that they might have *Mtb* infections, which could be the reason for lower *VDR* gene expression than negative IGRA household contacts, which may result in low levels of cathelicidin that are not sufficient to eliminate TB completely but may still be able to reduce excessive multiplication. Thus, its expression may be relatively higher when compared to active TB patients but lower when compared to IGRA-positive household contacts.

In this study, positive IGRA was found in 61.64% (45/73) of household contacts. This prevalence was almost similar to the study in Uganda (68.42%), also included in the list of 30 highly endemic nations with TB/HIV (Jones-López et al., 2013). In comparison to previous research in South Koreas and Mongolia, however, the prevalence of positive IGRA in our study was relatively high (42.6% and 48.2%, respectively) (Eom et al., 2018; Gurjav et al., 2019). The difference in the prevalence of positive IGRA among household contacts may be due to several things, including differences in the character of the recruited contact sample, such as how close the contacts are to the index case, the immunity status of the contact, and other factors such as the infectious

rate of the index case (Eom et al., 2018). In this study, most of the household contacts recruited were spouses (50.69% (37/73)) of positive AFB-index cases, and 75.67% (28/37) of them have positive IGRA results. Among the 45 positive IGRA HC, 28 (62.2%) were spouses. This study represented an immense rate of TB infection in the spouse of index cases. This was consistent with the investigation by Crampin et al., which revealed that the couples are in close contact having significant TB risks (Crampin et al., 2011).

A positive IGRA suggests the synthesis in the region of the Difference1 segment (RD1) in response to two *Mtb*-specific antigens, namely early secretive target-6 (ESAT-6), and culture filtrate protein 10 (CFP-10) (Zellweger et al., 2020). Currently, it is used to detect TB infection and is included in the diagnostic pathway for latent TB infection (Chapman and Lauzardo, 2014; Kementerian Kesehatan Republik Indonesia, 2020). IGRA is not recommended as a diagnostic tool to differentiate active and latent TB (Zellweger et al., 2020). Therefore, in this study, the IGRA was carried out on household contacts who did not have any clinical symptoms of TB and without a previous history of TB, so the IGRA here aims to determine whether the household contacts have been latently infected with TB or not. The immunological response in latent TB hosts can control the infection but is not sufficient to eradicate the mycobacterium (Gideon et al., 2011). The prevalent assumption is that the bacilli in latent TB stop growing and enter an immobile phase because of the "strong" immune response created by the host, eventually becoming non-replicable while maintaining the capacity to develop further under advantageous conditions in granulomas (Gideon et al., 2011; Ehlers and Schaible, 2013; Rao et al., 2019).

Several studies suggest that *VDR* expression is also influenced by the *VDR* polymorphism (Ogunkolade et al., 2002; Decker and Parker, 1995; Jurutka et al., 2000; Panda et al., 2019), thus possibly contributing to the development of TB disease (Xu and Shen, 2019; Cao et al., 2016; Panda et al., 2019; Lee and Song, 2015). Current research show varying results in various populations and several meta-analysis of common reported *VDR* polymorphism have attempted to conclude the association of VDR polymorphisms with the incidence of TB (Xu and Shen, 2019; Cao et al., 2016; Huang et al., 2015; Gao et al., 2010; Chen et al., 2013). In this study, we tried to analyze the polymorphisms in the 3' UTR *VDR*, SNP rs1157413 G > C, which has not been investigated much, especially in tuberculosis patients. Since it was located in intron 8, rs11574113 do not affect the sequence of VDR protein. The rs11574113 G/C variant has been reported to be associated with a reduced risk of colorectal cancer only in those with high plasma vitamin D concentrations. (Budhathoki et al., 2016). Meanwhile, in this study, the rs11574113 G/C variant had a significantly higher *VDR* gene expression than G/G only in the IGRA HC negative group (Fig. 2). Overall, this study observed significantly higher *VDR* gene expression in the G/G and G/C when compared to C/C genotype groups (Fig. 1). Besides, our study attempted to estimate the impact of SNP rs11574113 G/C variant on *VDR* gene expression by controlling for several variables and found that G/G genotype and G/C genotype were associated with higher *VDR* gene expression compared to C/C genotype. However, this needs to be investigated further considering that the C/C genotype in this study was only found in two samples and was limited to the PTB group. Furthermore, it is necessary to conduct in a larger size to confirm this finding, whether this SNP may have a direct effect on *VDR* expression or transcripts stability, or are in disequilibrium with other functional polymorphisms that regulate VDR activity, such as single(A) repeat in exon 9 (Whitfield et al., 2001) or affect transcript stability, such as the *VDR TaqI*, also located on exon 9 (Hussain et al., 2019). In another study of TB, it was found that the A allele of rs11574113 had a protective effect against TB (Hu et al., 2016), but in our study, we did not observe the presence of the A allele in any of the participants.

Several previous studies have studied the correlation of *VDR* gene expression with vitamin D levels in the blood. A recent randomized controlled trial in monozygotic twins studied increased VDR gene expression and 25-hydroxyvitamin D levels after 60 days of vitamin D (cholecalciferol) supplementation. In addition, the study observed that increasing levels of 25-hydroxyvitamin to levels greater than 50 ng/mL contributed to increased expression of the *VDR* gene (Medd et al., 2020). A US cohort involving patients with sickle cell anemia found that decreased vitamin D levels were associated with decreased expression of the *VDR* gene (Han et al., 2018). Contrary to these findings, a study of patients with oral neoplasms in India revealed an increase in *VDR* expression but not significantly compared to controls, while 25-hydroxyvitamin D levels expressed in vitamin D scores were found to be significantly lower in the case group of oral neoplasms than controls (Anand et al., 2017). Although this study did not measure the correlation of *VDR* gene expression with 25-hydroxyvitamin D levels, it can be seen that there was an opposite effect between *VDR* gene expression and 25-hydroxyvitamin D levels. Meanwhile, there were also studies showing that there was no correlation between *VDR* gene expression with 25-hydroxyvitamin D levels but with CYP24A1/CYP27B1 expression in epilepsy patients (Mazdeh et al., 2018). This study indicates that there may be a different correlation between 25-hydroxyvitamin D levels and VDR gene expression in various diseases and populations, or indeed there may not be a simple correlation between 25-hydroxyvitamin D levels and *VDR* gene expression as with 1,25 dihydroxyvitamin D levels described previously (Ogunkolade et al., 2002).

As for tuberculosis, downregulation of *VDR* gene expression has been associated with changes in 1,25-dihydroxyvitamin D levels in a number of studies. Selvaraj et al. revealed that in TB patients, *VDR* gene expression was downregulated which was thought to be caused by high levels of 1,25-dihydroxyvitamin D (Selvaraj et al., 2009). On the other hand, other studies have shown that levels of 1,25-dihydroxyvitamin D increase *VDR* mRNA production, stabilize *VDR* mRNA or protect VDR from degradation thereby increasing overall VDR counts (Kongsbak et al., 2013; Liu et al., 2006). 1,25-dihydroxyvitamin D plays a role in converting the VDR to a functionally active protein that can bind to RXR and to specific gene sequences and coregulators required for modulation of gene expression (Pike and Meyer, 2011; Pike and Meyer, 2012; Haussler and Whitfield, 2013). Therefore, the availability of 1,25-dihydroxyvitamin D is a prerequisite for VDR activity. During immune reactions, the most likely source of 1,25-dihydroxyvitamin D is primarily the endogenous production of its precursor molecule, 25-dihydroxyvitamin D. Several studies on immune cells have revealed that 25-hydroxyvitamin D can be utilized and subsequently converted to 1,25-dihydroxyvitamin D via the action of the enzyme CYP27B1 (Jeffery et al., 2012). The increased synthesis of 1,25-dihydroxyvitamin D under these circumstances may lead to the use of available 25-hydroxyvitamin D (Selvaraj et al., 2009). Because 1,25-dihydroxyvitamin D has a lower half-life and is used for various metabolic processes, active pulmonary TB patients may suffer from a lack of substrate for the synthesis of the active metabolite, including 25-hydroxyvitamin D (Selvaraj et al., 2009).

We tried to assess the association of *VDR* gene expression with active pulmonary tuberculosis and the outcome of positive IGRA. After adjustments to other variables, the multivariate analysis indicated an association between *VDR* gene expression and active TB susceptibility (Table 5). However, there was no association between the *VDR* gene expression and the IGRA positivity result in household contact even after adjusted with other variables (Table 6). The factors that showed an association with the positivity of IGRA were BMI and the relationship to the index case (Table 6). BMI also appears to be significantly associated with active TB susceptibility (Table 5). This result may suggest two possibilities. First, the greater the BMI, the lower the chance of contracting TB, which is consistent with prior findings (Kim et al., 2018; Kumaratne et al., 2017). A high BMI would seem to protect the immune system. Adiposive tissue is known to secrete leptin and TNF, an inflammatory mediator that helps defend the host from TB infection (Dorhoi and Kaufmann, 2014). Second, TB infection itself may cause weight loss, thus decreasing nutritional status (Cegielski and McMurray, 2004).

There were several limitations in this study. We realize that the sample used in this study is not large, but we hope that the picture obtained in this study can be developed with a larger number of samples in the future to obtain more adequate conclusions. In our study, we tried to involve variables such as smoker status, the relationship of household contacts with index cases, and the intensity of AFB which has been associated with the spread of *Mtb* germs at home (Crampin et al., 2011; Guwatudde et al., 2003; Sepkowitz, 1996). However, we did not examine other factors such as blood vitamin D levels, dietary factors or vitamin D supplementation that might influence *VDR* gene expression. A more comprehensive study including other components involved in the vitamin D metabolic pathway would certainly provide more adequate results in assessing the role of VDR in TB susceptibility. Likewise, analyses involving interactions between genes or even genes and the environment will certainly provide a better understanding of genetic influences on individual susceptibility to TB.

To date, there is still a lack of studies investigating the expression of the *VDR* gene in people who are latently infected with TB. The literature has explained how the role of VDR in eliminating *Mtb* through the vitamin D-dependent pathway (Chung et al., 2020). Several previous studies have also reported lower vitamin D (25-hydroxyvitamin D) concentrations in LTBI individuals than in active TB patients (Hong et al., 2019; Esteve Palau et al., 2015). The present study found that the expression of the *VDR* gene in IGRA-positive household contacts was higher than in active TB, and it may reflect the VDR protective efforts against TB in household contacts at the time of the examination. Whether with adequate VDR, cathelicidin as a mycobactericidal is continuously produced in sufficient quantities, or VDR may be involved in maintaining *Mtb* in the non-replicative phase is still not clearly understood. The specific role of VDR involved in the latency phase needs further investigation.

5. Conclusion

To conclude, *VDR* gene expression may be associated with active pulmonary tuberculosis (PTB) but not with IGRA positivity in this study. The *VDR* gene expression was downregulated in PTB rather than positive and negative IGRA HC. The high expression of the *VDR* gene may be influenced by *VDR* SNP rs11574113. This result may suggest a protective role of VDR in the progression of PTB. This study also found that among spouses of IGRA-based PTB patients, in comparison with other family members spouses are at greater risk of PTB infection. Spouses of PTB patients need adequate protection so that existing *Mtb* infections will not progress to the active phase.

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CRediT authorship contribution statement

Najdah Hidayah: Methodology, Software, Investigation, Writing-original draft preparation **Irawaty Djaharuddin:** Conceptualization, Writing - Reviewing and Editing, Supervision **Ahyar Ahmad:** Conceptualization, Validation, Writing - Reviewing and Editing, Supervision **Agussalim Bukhari:** Methodology, Supervision, Validation **Ilhamjaya Patellongi:** Methodology, Software, Validation **Nur Ahmad Tabri:** Investigation, Validation, Writing- Reviewing and Editing **Susana Agus:** Investigation, Validation, **Subair Subair**: Investigation, Writing - Reviewing and Editing **Irda Handayani:** Investigation, Writing - Reviewing and Editing **Andi Tenriola:** Investigation, Methodology, Software **Handayani Halik:** Methodology, Software, Validation **and Muhammad Nasrum Massi**: Conceptualization, Validation, Writing - Reviewing and Editing, Supervision.

Declaration of competing interest

None.

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