Correlation between Vitamin D Receptor Gene FOKI and BSMI Polymorphisms and the Susceptibility to Pulmonary Tuberculosis in an Indonesian Batak-ethnic Population

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ABSTRACT

Tujuan: untuk mengetahui peran polimorfisme Fokl dan Bsmi gen reseptor vitamin D (RVD) pada kerentanan terhadap tuberculosis (TB) paru suku Batak di Indonesia. Metode: disain penelitian adalah kasus kontrol berpasangan, dengan 76 pasien TB paru sebagai kasus dan 76 orang sehat yang tidak menderita TB paru sebagai kontrol. Polimorfisme gen RVD dianalisis menggunakan teknik PCR-RFLP. Hasil: frekuensi genotip Fokl adalah FF 35.5%, Ff 55.3%, ff 9.2% pada kelompok TB paru dan FF 39.5%, Ff 44.7%, ff 15.8% pada kelompok kontrol. Frekuensi genotip Bsmi adalah BB 8%, Bb 68.4%, bb 23.7% pada kelompok TB paru dan BB 22.6%, Bb 73.7% dan bb 73.7% pada kelompok kontrol. Tidak ada hubungan yang bermaikan antara genotip Fokl dan kerentanan TB paru (OR 1.39, 95% CI: 0.69-2.77 untuk genotip Ff dan OR 0.64, 95% CI: 0.22-1.86 untuk genotip ff). Pada polimorfisme Bsmi, ada hubungan yang bermaikan antara genotip Bsmi dan kerentanan terhadap TB paru. Genotip bb berhubungan dengan penurunan risiko terhadap terjadinya TB paru (OR 0.22, 95% CI: 0.11-0.45). Kesimpulan: pada populasi Indonesia etnik Batak, tidak ada hubungan antara polimorfisme Fokl gen RVD dan kerentanan terhadap TB paru. Pada polimorfisme Bsmi gen RVD, genotip bb berhubungan dengan penurunan risiko terhadap TB paru.

Kata kunci: tuberkulosis paru, polimorfisme, gen reseptor vitamin D, Batak, Indonesia.

ABSTRACT

Aim: to explore the role of Fokl and Bsmi polymorphisms in the VDR gene in the susceptibility to pulmonary tuberculosis (PTB) in an Indonesian Batak ethnic population. Methods: matched case-control study was conducted on 76 PTB patients and 76 healthy normal control. Genetic polymorphisms of Vitamin D Receptor (VDR) gene were analysed using PCR-RFLP. Results: the frequencies of Fokl genotypes were FF 35.5%, Ff 55.3%, ff 9.2% for PTB patients and FF 39.5%, Ff 44.7% and ff 15.8% for normal control. The Bsmi genotypes frequencies were BB 8%, Bb 68.4%, bb 23.7% for TB patients and BB 22.6%, Bb 73.7% and bb 73.7% for control. There was no significant association between Fokl genotype and PTB (OR 1.39, 95% CI: 0.69-2.77 for Ff genotype and OR 0.64, 95% CI: 0.22-1.86 for ff genotype). There was a significant association between Bsmi...
genotype and PTB; the bb genotype was associated with a decreased risk to PTB (OR 0.22, 95% CI: 0.11-0.45). Conclusion: in Indonesian Batak ethnic population, there was no association between FokI polymorphism of VDR gene with host susceptibility to PTB. There was a significant association between BsmI polymorphism of VDR gene; bb genotype was associated with a decreased risk to PTB.

Key words: pulmonary tuberculosis, polymorphisms, vitamin D receptor gene, Batak, Indonesia.

INTRODUCTION

Pulmonary tuberculosis (PTB) is still a public health problem in the world. About 1/3 of people in the world are infected with Mycobacterium tuberculosis, but only 10% of them develop clinical disease. Susceptibility to disease after mycobacteria infection is influenced by agent, environmental and host genetic factors. Many studies have strongly indicated that genetic factor plays an important role in the disease development. Vitamin D receptor gene is one of the recently interesting candidate gene.

Besides a calcitriol, phosphor and bone metabolism regulator, vitamin D also has nonskeletal function. Many studies showed an association between vitamin D deficiency and certain diseases such as systemic lupus erythematos, diabetes mellitus, tuberculosis, etc. In tuberculosis infection, vitamin D can activate macrophage to restrict Mycobacterium tuberculosis intracellular growth. This effect is achieved through binding to vitamin D receptor (VDR) in macrophages, hence activate cathelicidin synthesis, and consequently eliminate Mycobacterium tuberculosis in phagolysosomes. Those process might be affected by polymorphisms in the VDR gene.

One of these polymorphisms is FokI, transition T to C (ACG-ATG) at the first of the two potential translation initiation sites in exon 2, and can be distinguished by RFLP using endonuclease FokI. If translation start at the first ATG site (individuals with T allele, designated f), VDR protein synthesized full-length (427 amino acids). In contrast, if the translation start at the second ATG site (individuals with C allele, designated F), the VDR protein lack the three NH2-terminal amino acids. Some studies indicated that transcription of the F allele is 1.7 more than f allele. F allele interacts with transcription factor IIB more efficiently and result in more potent VDR protein transcription.

The second polymorphism in this study is BsmI, which located at intron between exon VIII and IX. The BsmI polymorphism influence transcription level, transcription stability and post modification transcription of VDR gene.

The potential roles of VDR FokI and BsmI polymorphisms in the development of PTB have been investigated in many ethnic group. The different results may be due to diverse ethnic background. Meta-analysis by Gao (2010) found that in Asians, subjects with ff genotype were more susceptible to TB and subjects with bb genotype have a decrease risk for TB. However, none of the polymorphisms was significantly related to TB among Africans or South Americans. Because the genetic effect may be different in various ethnic group, we undertook this study in Indonesian Batak-ethnic population, to know the role of FokI and BsmI polymorphisms VDR gene in the susceptibility to pulmonary tuberculosis.

METHODS

This is a matched case-control study with FokI and BsmI vitamin D receptor gene polymorphisms as independent variable and pulmonary tuberculosis as dependent variable. Sample size was determined by using matched case-control formula and number of sample was 76 subjects for each group. Cases were found by consecutive sampling and controls were subjects from healthy workers. This study has been approved by the Ethics Committee of the Faculty of Medicine, University of Sumatera Utara, Medan, Indonesia.

Subjects

Cases were pulmonary tuberculosis patients were recruited from several TB services in Medan city, Indonesia from November 2012 to April
Depending on the digestion pattern of FokI polymorphism, individuals were scored as FF, when homozygous for the presence of the FokI site (169 bp and 96 bp), FF when homozygous for the absence of the FokI site (265 bp), or Ff in case of heterozygosity (265 bp, 169 bp and 96 bp). The digestion pattern of BsmI polymorphism were BB for the presence of BsmI site (175 and 650 bp), BB when homozygous for the absence of the BsmI site (825 bp), and Bb in case of heterozygote (825, 175 and 650 bp).

**Statistical Analysis**

The genotype frequencies of each SNPs were compared by Mc Nemar Chi-square test as appropriate. The strength of the association between VDR FokI, BsmI polymorphisms and TB risk was evaluated by calculating odds ratio (OR) with 95% confidence interval (95% CI). Conditional logistic regression was performed to calculate the odds ratio. Data were managed and analysed using Epi Info. Hardy-Weinberg equilibrium test was done in case and control groups for FokI and BsmI polymorphisms using the web tool HWE Testing calculator, available on line[1]; p<0.05 was considered as a significant disequilibrium.

**RESULTS**

The characteristics of PTB patients and normal control are summarized in Table 1. Sex, age and ethnic characteristics between PTB patients and controls were matched.

The results of VDR genotyping for PTB patients and healthy controls are summarized in Table 2. Pulmonary tuberculosis patients and

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases - n (%)</th>
<th>Control - n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>53 (69.7)</td>
<td>53 (69.7)</td>
</tr>
<tr>
<td>Female</td>
<td>23 (30.3)</td>
<td>23 (30.3)</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16-25</td>
<td>22 (28.9)</td>
<td>22 (28.9)</td>
</tr>
<tr>
<td>26-35</td>
<td>32 (42.1)</td>
<td>32 (42.1)</td>
</tr>
<tr>
<td>36-45</td>
<td>14 (18.4)</td>
<td>14 (18.4)</td>
</tr>
<tr>
<td>46-55</td>
<td>8 (10.5)</td>
<td>8 (10.5)</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Batak ethnic</td>
<td>76 (100)</td>
<td>76 (100)</td>
</tr>
</tbody>
</table>
healthy controls had similar distribution for FokI genotype and allele frequencies (p>0.05). On the BsmI genotype and allele frequencies, there was a significant difference between PTB patients and healthy controls (p<0.001). The genotypes of FokI polymorphism in cases and controls group were in Hardy-Weinberg equilibrium (P>0.05) and BsmI polymorphism in controls group as well. The BsmI polymorphism in cases group was not in Hardy-Weinberg equilibrium (p<0.05).

**Table 2.** Allele frequencies and genotype of VDR gene FokI and BsmI polymorphisms in PTB cases and controls

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Cases n (%)</th>
<th>Controls n (%)</th>
<th>p</th>
<th>HWE in cases X² (p)</th>
<th>HWE in controls X² (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FokI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotype FF</td>
<td>27 (35.5)</td>
<td>30 (38.5)</td>
<td>0.311</td>
<td>2.67 (&gt;0.05)</td>
<td>0.21 (&gt;0.05)</td>
</tr>
<tr>
<td>FF</td>
<td>42 (55.3)</td>
<td>34 (44.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ff</td>
<td>7 (9.2)</td>
<td>12 (15.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>76 (100)</td>
<td>76 (100)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele frequencies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>96 (63.2)</td>
<td>94 (61.8)</td>
<td>0.816</td>
<td></td>
<td></td>
</tr>
<tr>
<td>f</td>
<td>56 (36.8)</td>
<td>58 (38.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>152 (100)</td>
<td>152 (100)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BsmI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotype BB</td>
<td>0</td>
<td>2 (2.6)</td>
<td>&lt;0.001</td>
<td>20.55 (&lt;0.05)</td>
<td>0.14 (&gt;0.05)</td>
</tr>
<tr>
<td>Bb</td>
<td>52 (68.4)</td>
<td>16 (23.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bb</td>
<td>24 (31.6)</td>
<td>56 (73.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>76 (100)</td>
<td>76 (100)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele frequencies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>52 (34.2)</td>
<td>22 (14.5)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>100 (65.8)</td>
<td>130 (85.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>152 (100)</td>
<td>152 (100)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HWE = Hardy-Weinberg Equilibrium; p<0.05 was considered as significant disequilibrium.

Relation between FokI and BsmI polymorphisms and pulmonary tuberculosis are summarized in Table 3. There was no significant association between FokI polymorphisms and PTB (OR 1.39, 95% CI: 0.69-2.77 for FF genotype and OR 0.64, 95% CI: 0.22-1.86 for ff genotype). On the BsmI polymorphism, the bb genotype was associated with a decreased risk to PTB (OR 0.22, 95% CI: 0.11-0.45).
DISCUSSION

Susceptibility to TB is the complex interaction between host, bacteria (agent) and environment. Host genetics is one of the host factors that can influence this susceptibility.

Comparisons with other researches showed that population or various ethnicities give different results. The non-significant relationship between VDR FokI polymorphism and PTB in this study were also found in Korea,14 Iran,15 Africa,16-19 South American,20 and spinal TB in India.21 Association between Ff genotype FokI polymorphism and susceptibility to PTB has been found in Han population in China,22 and association between FF genotype and susceptibility to PTB was found in Indian males.23 A meta-analysis in China on Han ethnic showed an association between ff genotype and susceptibility to PTB.24 Other meta-analysis from 13 studies showed association between ff genotype and susceptibility to PTB25 as well, and another meta-analyses from 29 case-control studies showed association between ff genotype and susceptibility to PTB in Chinese but not for other ethnicities.26 Another meta-analysis showed that ff genotype was associated with TB susceptibility among Asian population.12

Studies from Africa,17,27 India,28 and Korea24 found no association between BsmI polymorphism of VDR gene and PTB. The bb genotype was protective factor to PTB and extra PTB in Turki.29 Studies from Iran found bb genotype28 and Bb+bb genotype29 was associated with susceptibility to PTB. Meta-analysis from 15 studies found b allele and bb genotype associated with decreased risk to TB, especially in Asian population.30 In this study, we found that bb genotype was a protective factor to PTB or associated with decreased risk to PTB.

Some explanations are possible for the different results of this study compared to others. Ethnicity factor plays an important role. This study was restricted to Batak ethnic Indonesian population only, to prevent genetic bias from ethnic influence. Ethnicity is found to be a very important factor on genetic function in pulmonary TB disease. This can be seen in the different distribution of FokI allele in the world among populations or ethnicities. Frequency of f allele is lower among African race (24%) compared to the Caucasians (34%) and Asians (51%). For BsmI polymorphism, the frequency of B allele is 7% among Asians, 36% among Africans and 42% among Caucasians.32 Another studies found the BsmI bb genotype frequency was 2% among Asians, 5% among African Americans and 17% among Caucasians.33

The difference in the definition of case and control in each study can also alter the results. Some studies presume the case group as negative acid fast bacilli bacteria sputum or extra pulmonary TB, whereas for the control group, some studies took blood samples from donor banks where history of exposure to TB is not known. Similarly, not all studies have conducted HIV tests to determine the HIV status of the subjects.34

The different results could also be caused by gene-environment interaction, gene-gene interaction and gene-agent interaction. A study on Indian Gujarat ethnic resides in London showed no association between FokI polymorphism with
TB, but together with vitamin D deficiency, if genotype is associated with susceptibility to TB. This study confirm gene-environment interaction.14 Gene-gene interaction has been shown in some studies. A single gene that is not associated with susceptibility to TB will show an association if combined with other genes.8,15 Gene-agent interaction showed association of a certain host gene with a certain strain of Mycobacterium tuberculosis.13,34

The latest study showed an epigenetic variation on VDR gene and this discrepancy is different among various ethnic groups. Epigenetic variation and VDR 1a4d polymorphism interrelate with TB susceptibility. This demonstrates that interaction of genetic variation (genotype) with epigenetic variation (epigenotype) must be considered for associated study on diverse populations.29 There are other factors that may influence the results. It is known that before vitamin D enters the macrophage, inactive vitamin D in serum could be bound to vitamin D binding protein (DBP) or in a free state. After binding to Toll-Like Receptor on macrophage, inactive vitamin D is converted to active form by CYP27b1 enzyme on mitochondria. Consequently, along with heat shock protein 70 (hsp70) and bcl-2 associated anathogene (BAG-1), active vitamin D and its receptor enter the nucleus and forms heterodimer with Retinoid X Receptor (RXR) hence bind to Vitamin D Response Elements (VDRE) which is controlled by vitamin D response elements binding protein (VDREBP) on gene target promoter of cathelicidin.2 Therefore, all that occur in transcription of cathelicidin can influence the results of the study.

Further studies are needed to know the role of vitamin D Receptor in susceptibility to TB. In order to understand the pathogenesis of pulmonary TB that is useful for therapy and prophylaxis. Ethnic-specific genetic associated with TB susceptibility may guide TB therapy and prophylaxis in an ethnic-specific manner.5

CONCLUSION

In this study which is conducted in an Indonesian Batak ethnic population, FokI polymorphism of VDR gene is not responsible for host susceptibility to PTB. On the BsmI polymorphism, bb genotype was associated with decreased risk to PTB. These results, particularly for bb genotype BsmI polymorphisms, may give additional explanation why some people are more resistant against TB than the others. On the other hand, persons whose genotype that may cause them to be more susceptible to TB should be more aware about things that influence their immunity like nutrition, lifestyle such as smoking, alcohol consuming, etc.

ACKNOWLEDGMENTS

This study was partly supported by Directorate General of Higher Education, Ministry of Education and Culture, Indonesia (Hibah Bersaing Scheme, No. 4267/UNS.1.R/KEU/2013).

REFERENCES


BY M Sinaga, Y Siregar, M Amin, S Sarumpaet. "Effect of smoking and alcohol consumption on pulmonary tuberculosis among Batak ethnic population in Medan, Indonesia", IOP Conference Series: Earth and Environmental Science, 2018