Gene expression and Levels of Plasma Protein Tyrosine Phosphatase Non-Receptor Type 22 (PTPN22)in Pulmonary Tuberculosis Patients and their Household Contacts in Makassar, Indonesia

Ibrahim A[1,3], Djaharuddin I[2], Hidayah N[1], Subair[1], Handayani I[1], Tenriola A[1], Massi MN[4,5*]

[1] Reseach in Postgraduate Program, Medical Faculty, Hasanuddin University, Indonesia, [2]Asistant Professor in Pulmonogy Department, Medical Faculty, Hasanuddin University, Indonesia, [3]Reseach in Community Center for Lung Health Makassar, Indonesia, [4]Professor in Hasanuddin University Medical Research Center, Medical Faculty, Hasanuddin University, Indonesia, [5]Professor Microbiology Department, Medical Faculty, Hasanuddin University, Indonesia

Abstract

Background: The protein tyrosine phosphatase non-receptor type 22 (PTPN22) gen,has been involved in the immune response to tuberculosis infection by affecting the inflammatory response and subsequent anti-microbial immunity. This study aims to determine the PTPN22 expression and differences in the levels of PTPN22 in pulmonary TB patients (PTB) with household contacts and healthy control.

Methods: We analyzed PTPN22 expression and the level of plasma PTPN22 from pulmonary tuberculosis patients (PTB), household contacts and healthy control using real time PCR and ELISA method.

Results: ThirtyPTB, 30 household contacts and 30 control were involved in this study. Analysis of the assocation of PTPN22 gene expression against TB showed that PTPN22 gene expression was 7.3 times upregulated compared to household contact and 12.1 times upregulated compared to healthy controls.Levels of plasma PTPN22 in PTB: 10.0620 ng/ml, in Household contact: 6.7923 ng/ml and in control: 4.4293 ng/ml. These values did not differ significantly between the patients, household contact and control.

Conclusion: Our study results found that PTPN22 gene expression is significantly increased in PTB than household contact and control.Levels of plasma PTPN22 in PTB patientsdid not differ significantly than in household contact and control.

Keywords: *PTPN22, Pulmonary tuberculosis, Gene expression, Real time PCR, ELISA, Level of plasma.*

Introduction

Pulmonary tuberculosis (TB), an infectious disease caused by *Mycobacterium tuberculosis* (Mtb). TB remains a major cause of morbidity and mortality in humans worldwide. One-third of the population worldwide is infected by Mtb. About 5-10% of them manifest clinically to be active TB[1,5,10,15.] World Health Organization in Global Tuberculosis Report 2019 revealed that there were an estimated 10 million new TB cases worldwide in 2018. TB is the top infectious killer in the world. There were 1.5 million people died from TB[2,3,11,12,18]. TB is the leading killer of people with HIV and a major cause of deaths related to antimicrobial resistance. Indonesia is one of the countries with the highest TB burden in the world. There were 845 thousands TB incidence (316 per 100 thousands population) and 98.3thousands (37 per 100 thousands population)[4,6,7,8,21]

Studies in United States revealed that 20-30% of the household contacts occured latent infection and about 10% became active TB[9,10,13,14,28]. A person susceptibility to TB infection and developing active TB is influenced by several factors including host genetics, host immunity, mycobacterium virulence and environment. The prevalence rate of TB was significant different among several ethnic minorities, therefore,

the differences in susceptibility to TB may be related to a genetic predisposition . Genetic predisposition as one of the host factors influencing risk factors for the development of TB[15,16].

Studies revealed the pivotal function played by cellular immunity in Mtb infection. The immune responses are implicated to control the infection through cytokine production and specific surface molecules interactions. T-cell activation is a crucial step in the immune response against Mtb. Several genes are reported to have been associated with TB pathogenesis, one of them is protein tyrosine phosphatase nonreceptor type 22 (PTPN22) [17,18,,23]. The PTPN22 gene were involved in maintaining the T cells in the resting stage. PTPN22 were also responsible for bringing back the activated T cells to the resting phenotype in the absence and presence of antigen. Phosphotyrosine phosphatases are involved in reversion of T lymphoblastic proliferation Tyrosine phosphorylation in T cells is regulated by phosphatase activity.

Several transmembrane molecules, like PD-1 (programmed death 1) and CTLA-4 (cytotoxic T-lymphocyte antigen 4), play an important role in downregulating signalling through the T-cell receptor (TCR). The PTPN22 is a cytosolic inhibitor of TCR signalling. The PTPN22 geneis located on chromosome 1p13.3-p13.1. This gene product is the intracellular protein tyrosine phosphatase known as Lyp and expressed in cells of the immune system, including dendritic, T and B cells. Lyp is expressed in cells of haematopoietic origin and has a variety of substrates, including Lck, Zap70, Valosine containing protein (VCP), Vav and TCRzeta, all of them are important players in T-cell signalling. The dephosphorylation of these substrates by LYP negatively modulates T-cell activation. Lyp forms a protein complex with the intracellular tyrosine kinase (protein tyrosine kinase) Csk. The Csk protein tyrosine kinase is a potent suppressor of T cell activation due to its ability to phosphorylate tyrosine residue at Src family kinases, thereby antagonizing the action of CD45, that mediates dephosphorylation of the inhibitory C-terminal SH2 domain of Lck. [19,1,4]

Numerous studies from several countries result, suggest that the PTPN22 gene affect susceptibility to TB. However, no research has been conducted in Indonesia about PTPN22 andTB [4,7,12]. The purpose of this study was to compare gene expression and levels of plasma PTPN22 in pulmonary tuberculosis patients and their household contacts in Makassar, Indonesia. The results of this study are expected to provide a better understanding of the relationship gene expression and levels of plasma PTPN22 with susceptibility TB.

Materials and Method

Research design and study subjects

We analyzed gene expression and the levels of plasma PTPN22 from pulmonary tuberculosis patients (PTB), household contact and control samples using real time PCR and ELISA method. QuantiFERON-TB Gold Plus (IGRA) was used to screening latent TB infection among household contacts. A total of 90 samples were collected in this study, consisting 30 pulmonary tuberculosis patients, 30 household contacts and 30 control. Pulmonary TB patients who were participants in this study were recruited from the Community Center for Lung Health Makassar, Indonesia, which is one of the referral health facilities for tuberculosis.

All TB patients were diagnosed based on clinical manifestations, chest radiograph, microscopic smear which was further confirmed through TB culture with MGIT medium. Inclusion criteria for TB patients in this study included new TB cases (no history of anti-tuberculosis drugs treatment), ages 18 years and over, willing to participate in this study by giving written consent and having positive smear results. The exclusion criterion was HIV-positive (SD Bioline). The inclusion criteria for household contact are those aged 18 years and over, have no clinical symptoms of TB, have no history of TB or anti-tuberculosis drugs, and stay at home with TB patients for at least 6 months and are willing to participate by giving written consent. We collected blood and sputum samples from 30 TB patients, blood samples from only 30 household contacts that met the criteria and 30 blood samples from healthy control. Positive sputum samples were decontaminated and continued with the culture process at the Tuberculosis Unit of the HUM-RC Laboratory (Hasanuddin University Medical-Research Center), Makassar, Indonesia. Blood samples were centrifuged at 4,400 rpm for 10 minutes at 250C to separate plasma samples. Plasma samples were stored at -200C before ELISA. Specifically from the contact sample, we examined IGRA with the QuantiFERON Gold Plus TB Test (Qiagen, Germany) according to the manufacturer's instruction manual [20,21,4,7]

Measurement levels of plasma PTPN22

Concentration PTPN22 was determined by the ELISA method using an ELISA Human PTPN22 kit (Diagnostics Biochem Canada Inc., Ontario, Canada) according to manufacturer's instructions. Linear curves are used to determine the concentration of PTPN22 samples from a calibration curve. **Measurement gene expression of PTPN22 by real timePCR**

Amplified complementery DNA (cDNA) from RNA exctracted blood sample by Reverse Transcriptase-PCR based on the method of Invitrogen. Using SuperScript First-Strand Synthesis System for RT-PCR. This cDNA strand stored in -20^{0}C until used for real time PCR. Measurement gene expression (up regulation or down regulation)PTPN22 bySYBR Green Dye using the real time PCR (qPCR) method according to manufacturer's instructions. Before amplified, a master mix was prepared by mixed 12.5 ml SYBR green dye, 0.5 ml cDNA and 0.5 ml forward primer (5'-ACAACTGTGGCTGAGAAGCCCA-3'), 0.5 ml reverse primer (5'-GTAGCTGGAATCCTCATCAGAGG-3') (each primer 5 pmol/ml) and 11.3 ml H_2O. The same procedure was carried out for the GAPDH gene as a control but using a different primer sequence. GAPDH forward primer (5'-CCTGCACCACCAACTGCCTTA-3') and GAPDH reverse primer (5'-GGCCATCCACAGTCTTCTGAG-3') [3,7,9,10]. The qPCR cycle with a condition of 50^{0}C for 2 minutes, 95^{0}C for 1 minute each1 cycle, denaturation 95^{0}C for 15 seconds then folowed by anneling 60^{0}C for 30 second and extension 72^{0}C for 30 seconds was repeated 40 times (cycles). The last cycle was the final extension at 72^{0}C for 10 minutes [1,4,9,15,17].

Statistical Analysis

All experimental data were analyzed using SPSS software (version 21.0, Chicago, IL, USA). P values <0.05 were considered statistically significant. Data obtained from ELISA results were analyzed and assessed for differences between the three groups (pulmonary TB patients, household contacts and control). The Chi-square test is used for the comparative analysis of nominal variables.Data (parametric) will be presented as mean ± SD (median) and will use statistical analysis to distinguish values between the three groups. Data were analyzed using the One-way ANOVA test which compared levels of plasma PTPN22 in the three groups.

Results

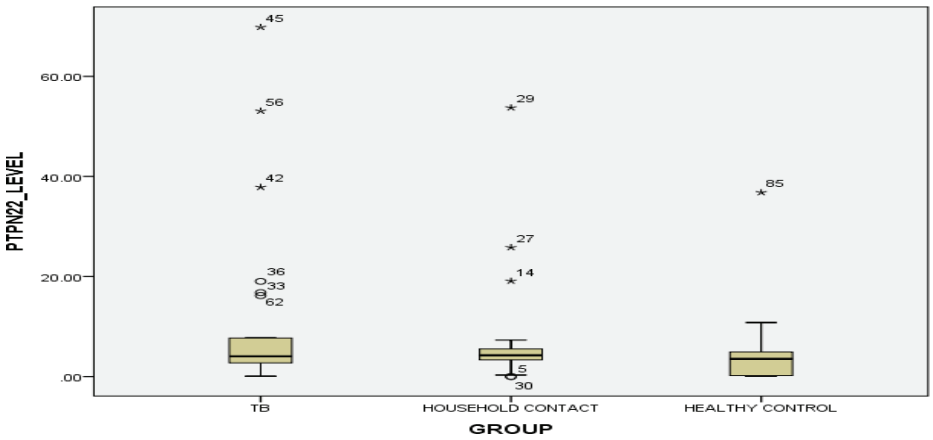
We analyzed a total of 30 TB patients, 30 household contacts and 30 healthy control for the PTPN22 gene expression and levels of plasma.

Table 1. Distribution of study participants according to the demographic characteristics

Variable	**TB patients n= 30**	**Household contact n=30**	**Healthy control n=30**	p-value
Gender Male Female	 17 (56.7%) 13 (43.3%)	 6 (20%) 24 (80%)	 15 (50%) 15 (50%)	0,009*
Age (years) 17-30 31-45	 7 (23.3%) 10 (33.3%)	 9 (30%) 13 (43.3%)	 3(10%) 12 (40%)	0,475*
46-60 > 60	11 (36.7%) 2(6.7%)	7 (23.3%) 1 (3.3%)	13 (47,2%) 2 (6,7%)	

* Chi-square test.

Table 1 shows the gender and age of study participants. There was a gender difference between groups (p= 0.009). There were more male subjects (56,7%) than female (43.3%) in the TB patients group. However, more female subjects (80%) than male (20%) in household contacts group. It can be seen that there was no age difference between the three groups (p=0,475).



Test of Annova p = 0,175.

Figure 1. Levels of plasma PTPN22 in units of ng/ml in TB patients group, household contacts and control.

PTPN22 levels were measured using ELISA. The lines on the boxplot indicate the median and the points show extreme values. PTPN22 levels in TB patients group: mean10.06 ng/ml withstandard deviation15.98 ng/ml. PTPN22 levels in household contacts group: mean 6.79 ng/ml with standard deviation 10,24 ng/ml. PTPN22 levels in control group: mean 4.43 ng/ml with standard deviation 6.68ng/ml. Data were analyzed using the One-way ANOVA test which compared PTPN22 levels in the three groups.

The mean value PTPN22 levels in TB patients compared to household contacts and control, did not differ significantly (p = 0,175).

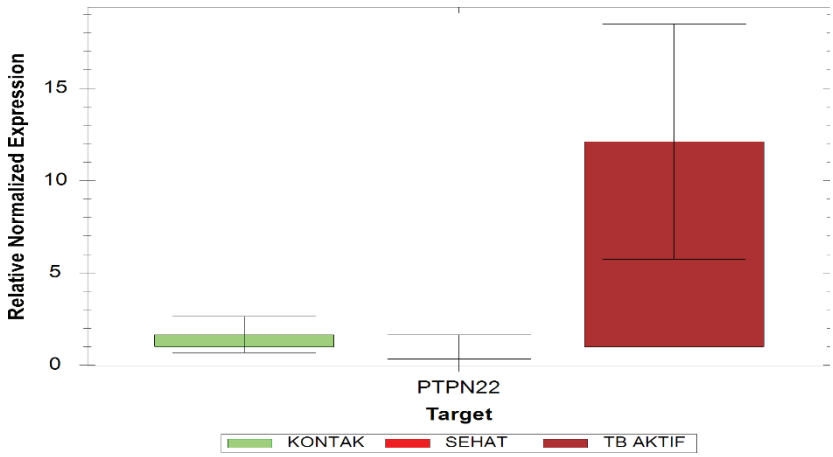


Figure 2. Gene Expression of PTPN22 in TB patients group, household contacts and control.

Gene expression of PTPN22 in TB patients group: 12.11629, household contacts group: 1.65801 and control1.00000.Analysis of the assocation of PTPN22 gene expression against TB showed that PTPN22 gene expression was 7.3 times upregulated compared to household contact and 12.1 times upregulated compared to healthy controls.

Discussion

In this study, from 90 participant, there were more male subjects in TB patients group. This was in accordance with the theory . We found that PTPN22 gene expression is significantly increased in PTB than household contact and control. Several studies analyzing the role of PTPN22 in TB show an association between

PTPN22 and susceptibility to active TB disease. Some of the earlier studies reported a significant association between PTPN22 and TB. These studies results suggest that PTPN22 gene may affect susceptibility to TB like in Colombian population, Moroccan population, Brazilian Amazon population and Chinese Uygur population[23,10,15,]

The mean of levels plasma PTPN22 gene on TB patients group was higher thanmean of levels plasma PTPN22 gene on household contacts and healthy control. But these values did not differ significantly levels plasma of the PTPN22 gene between thethree groups [24,10,28]. Several studies resultsalso found that PTPN22 gene is not associated with the susceptibilty to TB, such as in Iranian population and Indian population[2,9,17,19]

There were differences in terms of genetic predisposition and serum levels in TB and non-TB subjects. It was likely due to geographical location, genetic differences and the presence of other disease conditions.A person susceptibility to TB infection and developing active TB is influenced by several factors including host genetics, host immunity, mycobacterium virulence and environment25,26 . Several factors were increased susceptibility TB, such as malnutrition, Diabetes mellitus and age by suppressed the immune system [27,4,22]

Conclusion

The PTPN22 gene expression is significantly increased in pulmonary TB patients than household contact and control. Patients with active TB have higher levels of plasma PTPN22, but did not differsignificantly, compared to household contacts and control.

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Conflict of Interest- None of the authors has competing interests.

Ethical Clearance- This research was approved by the Research Ethics Commission of the Faculty of Medicine, Hasanuddin University Makassar, South Sulawesi, Indonesia (No. 583/H4.8.4.5.31/PP36-KOMETIK/2018), and all research subjects gave written informed consent.

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