Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 14043-14048
Received 25 April 2021; Accepted 08 May 2021.

Plasma Butyrophilin-Like-2 (Btnl2) Profile Associated with Pulmonary Tuberculosis (Tb) Active and Latent TB Infection

Subair[1], Halik H[2,3], Ahmad A[4], Djaharuddin I[5], Handayani I[5], Tenriola A[1], Ibrahim A[1,6], Najdah H[1], and Massi MN[2,7*]

[1] Postgraduate Program, Medical Faculty, Hasanuddin University, Indonesia

[2] Hasanuddin University Medical Research Center, Medical Faculty, Hasanuddin University, Indonesia

[3] University Mega Rezky, Makassar

[4] Chemistry Department, Mathematic and Natural Science Faculty, Hasanuddin Univesity, Indonesia

[5] Pulmonogy Department, Medical Faculty, Hasanuddin University, Indonesia

[6] Community Center for Lung Health Makassar, Indonesia

[7] Microbiology Department, Medical Faculty, Hasanuddin University, Indonesia

ABSTRACT

Tuberculosis (Tb) is one of the most common causes disease and mortality in humans. BTNL2 gene, which is a gene that is at the border area between MHC class II and class III are associated with autoimmune diseases and infections such us tuberculosis. The study aim to understand the characteristics of different TB infection immunologistsatus better and to identify potential immune biomarkers that can distinguish the active disease from latent infection. In this study, we analyzed the level of BTNL2 from tuberculosis patients with BTA positive (ZN staining), their household contact and healthy as a control samples using ELISA method. For household contact and control sampel we done screening latent TB infection by using QuantiFERON-TB Gold Plus (IGRA). The results of an examination of BTNL2 levels showed that there were significant differences between BTNL2 levels in the active Tb, latent Tb, and healthy groups. BTNL2 Level used as a biomarker for the identification of pulmonary TB patients and LTBI from plasma specimens.

Keywords: Tuberculosis, BTNL2, IGRA, LTBI

INTRODUCTION

About 1/3 of the world's population carries the disease but has no symptoms (known as latent infection), but about 10% of these people are likely to develop active disease during their lifetime and become capable of transmitting bacteria (WHO, 2017). Latent tuberculosis is defined as an infection of Mtb that persists in the macrophage without replication, but has a latent ability and can cause active disease if there is an impaired immune response (Ahmad, 2011).

However, many patients do not have clear underlying risk factors, and now there is conclusive evidence that individual variability in tuberculosis susceptibility partly determined by the host gene (Bellamy, 2000; Johnson et al., 2007). A new concept that shows the predisposition of genetic effects on the reactivation of TB has widely studied in recent years. Several genes related to

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 14043-14048
Received 25 April 2021; Accepted 08 May 2021.

susceptibility to Tb disease have identified, one of which is the BTNL2 gene, which is a gene that is at the border area between MHC class II and class III.

BTNL2 is a member of the butyrophilin family and immunoglobulin superfamily. The BTNL2 gene is ˜170 kb from human leukocyte antigen (HLA) -DRB1 on the MHC haplotype reference sequence (NCBI35), at the border between MHC class II and class III regions, and a strong connection with this locus. It is thought to adopt the structure of a type 1 transmembrane protein with two extracellular immunoglobulin (Ig) Domain V and one IgC domain and contain homology to the B7 molecule (Stammer, 2000). These receptors have shown to be important in the initiation and stopping of cellular immune responses through interactions with CD28 ligands and protein-related cytotoxic T lymphocytes (C. M. Jonhson et al. 2006). Research conducted by Nguyen et al (2006) found that BTNL2 characterization produced BTNL2 fusion protein in mice that significantly affected the expression of B cells and T cells after activation. BTNL2 inhibits T cell proliferation and activation of TCR, NFAT, NF-B, and AP-1 signaling pathways.

An understanding of the balance between activating and suppressing molecules has led to the development of new therapies to block excessive activation or to utilize an increase in suppression mechanisms in expression, especially in antigen-presenting cells (APC). The aims of this to understand the characteristics of different TB infection immunologistsatus better and to identify potential immune biomarkers that can distinguish the active disease from latent infection. We analyzed the BTL2 levels from subjects with active pulmonary TB, latent pulmonary TB and healthy people.

METHODS

This research conducted for one year in Makassar, South Sulawesi. Sampling was carried out at the Makassar Center for Community Lung Health (BBKPM) and in the homes of the research subjects; then, the sample processing is carried out at the HUM-RC Laboratory of the UNHAS Hospital, Makassar. Samples from this study were sputum and blood from TB patients and blood samples from household contacts from patients who met the study criteria obtained from patients who visited BBKPM Makassar. Patient data were collected based on questionnaire/interview results and medical records of pulmonary TB patients.

Data on household contact obtained from questionnaire / interview results. Data in the form of age, sex, medical history (including a history of illness and treatment), smoking history, and socio-economic conditions. In Tb patients, sputum samples were taken, and acid-resistant Basil was examined using the Ziehl Nelsen staining method and culture and DST tests. For contact and control groups, blood was drawn for IGRA (latent TB) examination using IGRA-TB Gold (Qiagen) according to the manufacturer's instruction manual. In all sample groups, BTNL2 levels were examined using the ELISA technique.

RESULTS

This study analyzes the expression of BTNL2 genes and their protein levels and their relationship with active tuberculosis (TB) and latent TB. The study attended by 59 subjects with new cases of pulmonary tuberculosis obtained from the Center for Community Lung Health (BBKPM) with positive smear results determined as active Tb groups. A total of 63 subjects were in the same household contact with active TB patients who met the inclusion criteria where a Quantiferon TB Gold (IGRA) examination was performed to determine the exposure of Mycobacterium tuberculosis (Mtb). The subjects with positive IGRA examination results included in the latent Tb group of 46 (73%) subjects. Further sampling also performed on 59 subjects who had no previous contact history with Tb patients as a healthy (normal) group. In all normal subjects, IGRA tests also performed, and 13 (22%) samples were obtained with positive IGRA results and 46 (78%) negative IGRA results.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 14043-14048
Received 25 April 2021; Accepted 08 May 2021.

Characteristic

The data characteristics of the subjects in this study include age and gender. Of the 181 samples studied, the number of male respondents was 63 (34.8%) people and women were 118 (65.2%). In the active Tb group, male subjects 17.7% were more numerous than females 14.9% In contrast to the other 2 groups, female subjects were more numerous than men.

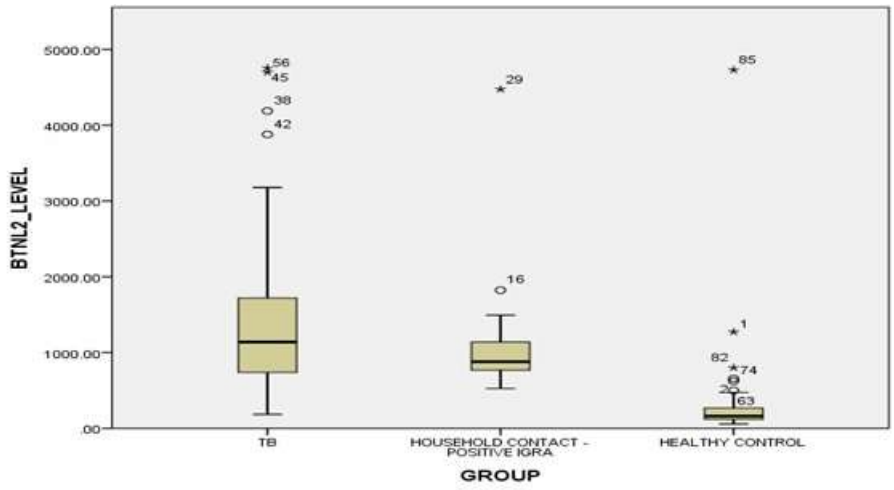
Table 1. Characteristics of study subjects in the active, Tb latent and healthy Tb groups

Characteristic	*Active TB*	*Household contact*	*Healthy*	*Total*	*p*
	n = 59	*n = 63*	*n = 59*		
Sex					
Man	32 (17.7%)	14 (7.7%)	17 (9.4%)	63 (34.8%)	.001
Women	27 (14,9%)	49 (27.1%)	42 (23.2%)	118 (65.2%)	
Age	41.7±11,9 (45)	39±16,5 (38.5)	44±14.1 (48)		.578
17 – 29	15 (8.3%)	20 (11%)	20 (11%)	55 (30.4%)	
30 – 29	31 (17.1%)	28 (15.5%)	22 (12.2%)	81 (44.8%)	
50 – 70	13 (7.2%)	15 (8.3%)	17 (9.4%)	45 (25%)	
IGRA status					
IGRA positive		46 (73%)	17 (27%)		.000
IGRA negative		13 (22%)	46 (78%)		
Relationship (Hc)					
child		9 (16.7%)			
parent		2 (6.7%)			
spouse		44 (50%)			
sibling		5 (16.7%)			
other		3 (10%)			

From the table above it can be seen that there is a relationship between gender and TB incidence (p = 0.001). In contrast, there was no significant difference in age between the three sample groups (p = .578). For ages, 44.8% of study subjects were between 30 - 49 years, of which the youngest was 17 years old, and the oldest was 70 years. The IGRA examination showed a

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 14043-14048
Received 25 April 2021; Accepted 08 May 2021.

positive relationship between IGRA and the TB case group (p = .000). These results indicate the fact that individuals who have contact facts have a greater risk factor (OR = 4.2) compared to individuals who have no contact history (healthy group). the household contact group, more in positive couples (husband or wife) (50%) compared to families, namely children (16.7%), parents (6.7%), relatives (16.7%), and others (10%).



Distribution of BTNL2 level in active Tb, LTBI and healthy patient

Figure 1. Boxplot (5-95%) BTNL2 levels in units of ng / ml in the active Tb group, Latent Tb (positive contact with IGRA) and healthy

Figure 1 above shows the difference in mean BTNL-2 levels between the three sample groups, where the highest average was in the active Tb group and the lowest was in the healthy group. Statistical test results (Kruskal-Wallis test) obtained p = 0.000, which showed that there were significant differences in BTNL2 levels both in the active Tb, latent Tb, and healthy groups.

DISCUSSION

To date, Tb is an infectious disease that is still a health problem in the world. The potential for infection with this disease is huge, especially in countries with high TB endemic cases. The human body is unlikely to avoid the environment that contains pathogens around it, and the body will always be threatened by pathogenic microbial boards, especially the Mtb bacteria which is transmitted through inhalation pathways. The host immune system plays a very important role in defense against Mtb infection both the cellular immune system and the humoral system, although the immune response in the body's defense mechanism against Mtb infection is still not fully understood.

Several studies related to the body's immune response to Mtb infection have developed, one of which is research on immune biomarkers based on the dynamics of T helper 1 (Th1) and Th2. Therefore, in this study, we carried out an immunological and molecular approach related to immune biomarkers in the body related to active Tb, latent Tb and healthy individuals; this is BTNL2.

In a study of 90 research subjects, data were obtained in which more active TB patients were in male subjects. This is in line with the WHO where more men diagnosed with TB with smear-positive than women (WHO, 2017).

Interferon-γ release antigen (IGRA) is emerging as a screening tool for the diagnosis of Infectious Latent Tuberculosis (LTBI). IGRA combines specific M. tuberculosis antigen which is not present

in the BCG strain and in most nontubercular mycobacteria, has high specificity for detecting M. tuberculosis infection (Lez-Fern et al, 2011). Prompt and appropriate treatment and early detection of active TB in patients is the basis of national TB control programs worldwide. However, identifying and treating latent TB infection (LTBI) in those at risk of developing active disease is critical to reducing the incidence rate and eliminating TB disease. In this study, the IGRA positivity rate was higher in individuals with a history of household contacts with active TB patients (73%) compared to individuals with no previous contact history (22%). It is estimated that two billion people are living with TB infection and it is a potential source of active TB in the future (Walzl et al., 2011). Several factors can trigger disease progression to become active from reactivation of infection which usually involves decreased immune response (Flynn & Chan, 2001; Van Crevel et al., 2002).

In particular, individuals with a history of home contact with people with active TB are at greatest risk for latent TB because they congregate and share the same airspace with TB patients for long periods of time (CDC, 2005). Furthermore, the likelihood of progression from latent TB to TB in household contacts is usually higher than in those without a history of household contact. Therefore, household contacts of TB patients are considered a high priority population for contact investigation (CDC, 2005).

The results of measuring BTNL2 levels using the Enzyme-Linked Immunoassay (ELISA) method in the active TB, latent and normal TB groups (Figure 1) obtained the highest average BTNL2 protein level in the active pulmonary TB patient group, namely 1112.1 ng/ml while the average BTNL2 protein levels were lowest in the healthy group, namely 331.6 ng/ml (4.2 ng/ml) and the healthy control group (2.14 ng/ml). The results of statistical tests obtained significant results ($p < 0.05$) which indicated that there was a relationship between increased levels of BTNL2 plasma protein and the incidence of TB disease.

Research conducted by Heather et al (2009) shows a relationship between BTNL2 and sarcoidosis, the impact of BTNL2 polymorphisms has shown in several inflammatory diseases. Tuberculosis and sarcoidosis show a phenotypic picture of granulomatous disease. Mycobacterium tuberculosis bacteria can induce the expression of BTNL2 gene susceptibility from sarcoidosis in monocyte-derived macrophages. Therefore BTNL2 was investigated as a gene candidate for tuberculosis in a case-control association study in a South African population (Möller et al., 2007).

In vitro, research conducted by Nguyen et al (2006) extracellular domain of BTNL2 mice was sufficient to inhibit T cell proliferation and cytokine production in response to anti-CD3 and other costimulatory molecules (Arnett et al. 2007; Arnett et al., 2009). IL-2 is one of the cytokines whose production is inhibited by BTNL2 and is very important for the survival and function of T cells. The addition of IL-2 to T cell culture in the presence of BTNL2 is able to restore some, but not all, proliferative capacity.

REFERENCES

1. Ahmad, S. (2011). Pathogenesis, immunology, and diagnosis of latent Mycobacterium tuberculosis infection. Clinical and Developmental Immunology, 2011.
2. Arnett, H. A., Escobar, S. S., Gonzalez-Suarez, E., Budelsky, A. L., Steffen, L. A., Boiani, N., & Viney, J. L. (2007). BTNL2, a butyrophilin/B7-like molecule, is a negative costimulatory molecule modulated in intestinal inflammation. *The Journal of Immunology, 178*(3), 1523-1533.
3. CDC. (2005). Guidelines for the Investigation of Contacts of Persons with Infectious Tuberculosis. Center of Disease Control and Prevention, Atalanta, USA.
4. Van Crevel, R., Ottenhoff, T. H., & Van Der Meer, J. W. (2002). Innate immunity to Mycobacterium tuberculosis. Clinical microbiology reviews, 15(2), 294-309.

5. Arnett, H. A., Escobar, S. S., & Viney, J. L. (2009). Regulation of costimulation in the era of butyrophilins. *Cytokine*, *46*(3), 370-375.

6. Flynn, J. L., & J. Chan. (2001). Tuberculosis: Latency and Reactivation. *Infect Immun. 69*: 4195-201.

7. Johnson, C. M., Traherne, J. A., Jamieson, S. E., Tremelling, M., Bingham, S., Parkes, M., ... & Trowsdale, J. (2007). Analysis of the BTNL2 truncating splice site mutation in tuberculosis, leprosy and Crohn's disease. *Tissue antigens*, *69*(3), 236-241.

8. Lez-fern, Á. F. G. Á. (2011). Tuberculin skin test and interferon-g release assay show better correlation after the tuberculin 'window period'in tuberculosis contacts. *Scandinavian Journal of Infectious Diseases*, *43*, 424-429.

9. Möller, M., Kwiatkowski, R., Nebel, A., van Helden, P. D., Hoal, E. G., & Schreiber, S. (2007). Allelic variation in BTNL2 and susceptibility to tuberculosis in a South African population. Microbes and infection, 9(4), 522-528.

10. Nguyen, T., Liu, X. K., Zhang, Y., & Dong, C. (2006). BTNL2, a butyrophilin-like molecule that functions to inhibit T cell activation. *The Journal of Immunology*, *176*(12), 7354-7360.

11. Bellamy, R. (2000). Identifying genetic susceptibility factors for tuberculosis in Africans: a combined approach using a candidate gene study and a genome-wide screen. *Clinical Science*, *98*(3), 245-250.

12. Walzl, G., Ronacher, K., Hanekom, W., Scriba, T. J., & Zumla, A. (2011). Immunological biomarkers of tuberculosis. *Nature Reviews Immunology*, *11*(5), 343-354.

13. World Health Organization. (2017). *Global tuberculosis report 2017*. World Health Organization.