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Research Article

REDUCTION OF TUMOR NECROSIS FACTOR-ALPHA AND INTERFERON-GAMMA CONCENTRATION ON TUBERCULOSIS WITH DIABETES MELLITUS AS A MARKER IN DECREASE IMMUNE SYSTEM

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ABSTRACT

Objective: This study aimed to determine the decreased immune response of tuberculosis (TB) with diabetes mellitus (DM) patients.

Methods: A total of 105 TB patients who were undergoing treatment at health centers and hospitals in Banda Aceh and Aceh Besar were included in this study. Data collection was carried out by interviewed to obtained demographic and respondent categories based on the diagnosis. Measurements of height and weight were also conducted to obtain body mass index data. 5 mL peripheral blood was taken from each respondent group into a TB with DM (TB+DM) and TB without DM (TB-DM). The blood tested usage tumor necrosis factor-alpha (TNF-α) level using enzyme-linked immunosorbent assay and interferon-gamma (IFN-γ) using IFN-γ release assay.

Results: The average concentration of both TNF-α and IFN-γ was higher in TB-DM group (TNF-a 5.2 pg/mL; IFN-g 1.5 IU/mL) than in TB+DM group (TNF-a 2.06 pg/mL; IFN-g 2.86 IU/mL). There were significant differences in TNF-α between the two groups but no significant differences in IFN-γ protein concentration.

Conclusion: The immune response of TB patients with DM symptoms was markedly reduced by the decreased expression of TNF-α and IFN-γ.

Keywords: Tuberculosis, Diabetes mellitus, Tumor necrosis factor-alpha, Interferon-gamma.

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INTRODUCTION

Pulmonary tuberculosis (TB) is an infectious disease caused by the bacterium *Mycobacterium tuberculosis* (M.tb). According to the WHO, in 2017, 10 million people worldwide are infected with TB, while in 2018, RISKESDAS reported the prevalence of pulmonary TB in Indonesia reaches 0.4% and 0.5% in Aceh [1]. Diabetes mellitus (DM) is a lifetime chronic disease that can reduce the quality of life. The prevalence of DM patients in Aceh is around 1.68% [1]. People with DM may increase the risk of developing TB by 1.5 times as research conducted in Jakarta and Bandung showed that the prevalence of DM in TB patients was around 17.1% and 11.6%, respectively [2].

The immune system responds to TB infection through tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ). Changes in the immune response also occur in people with DM, which will affect the susceptibility of DM patients accompanied by TB. Research by Lachamandes concluded that elevated blood glucose would increase TNF-α secretion in macrophages in TB patients. In addition, the phagocytosis ability of monocytes decreases on TB with DM (TB+DM) [3]. Meenakshi reported a study in India, where the production of IFN-γ was reduced in the TB group with DM [4]. In contrast, Kumar *et al*. showed the increased expression of CD4 in the IFN-γ-secreting T cells in TB patients with DM [5].

Aceh is one of the provinces in Indonesia, which has a high proportion number of TB and DM, andthis factor increases the difficulty of breaking the chain of TB contagious. The high rate of DM causes the possibility of individuals to be more susceptible to TB. The aim of this research is to observe the dynamic of an immune response in DM patients, which may contribute to the susceptibility of TB infection in Aceh Province.

METHODS

This research did 6 months in Banda Aceh and Aceh Besar. The samples in this study were patients with TB that treated in health centers in Banda Aceh and Aceh Besar in 2018. Samples of the study included 105 people who were willing to take part in the study (signed informed consent). Samples consisted of 55 people having TB without DM (TB-DM), 51 people having TB+DM. DM diagnosis determined based on the results of the HbA1c examination ≥6.5%. This study received ethics approval from the Health Research Ethics Commission, Health Research, and Development Agency No: LB.02.01/KE.162/2018.

Data collection carried out by interviewed to obtained demographic data and respondent categories data based on the diagnosis; measurement height and weight to got body mass index (BMI) data; and laboratory check to obtained a protein concentration of TNF-α and proteins IFN-γ serum.

The examination of TNF-α protein was conducted by the usage of the enzyme-linked immunosorbent assay (ELISA) sandwich test that used human TNF-α ELISA kit with product catalog number E-EL-H0109. A total of 100 µL of the serum added to the ELISA well, then incubated for 90 min at 37°C. After the liquid had been removed, 100 µL biotinylated detection Ab added and incubated for 1 h at 37°C. Then, it washed with wash buffer and added 100 µL horseradish peroxidase conjugate working solution and incubated for 30 min at 37°C. After the solution had been removed and washed, added 90 µL to the substrate and incubated for 15 min at 37°C. Then, a stop solution was added, and the results were read at a wavelength of 450 nm.

The examination of the protein concentration of IFN-γ was performed by usage the IFN-γ release assay (IGRA) with Quantiferon TB Gold Plus (QFT-Plus) blood collection tubes (product catalog number 004 716 361) and Quantiferon AKS-TB Gold Plus (QFT-Plus) 2 plate ELISA kit

(product catalog numbers AKS-004716074). A total of 1 mL of blood was put into the four IGRA test tubes that named nil tube, TB1, TB2, and mitogen tubes, and then incubated for 24 h at 37°C. Furthermore, plasma is separated from blood cells and followed by ELISA test. A total of 50 μL working strength conjugate was put into the ELISA well and 50 μL of sample or standard solution added and incubated for 120 min at 22°C. Then, the samples washed as much as 6 times, and 100 μL of the substrate was added and incubated for 30 min at 22°C. Finally, a 50 μL stop solution is added, and the results are read at a wavelength of 450 nm.

SPSS version 23 usage for analysis, univariate analysis performed to determine the categories of respondents based on the diagnosis. Bivariate analysis (Chi-square test and Mann–Whitney test) was also undertaken to examine the relationship between demographic status with the condition of DM in TB patients and differences in the averaged concentration of TNF-α and IFN-γ in the TB+DM group with TB-DM. The significance value was $p<0.05$.

RESULTS

This study included 105 respondents, with the dominant gender was male. For the TB+DM group, most respondents were in the age range between 46 and 60 years, while in the TD-DM group, most were in the age range between 31 and 45 years. Therefore, based on the Chi-square test, there were significant differences in age between the two groups. The education level of most respondents in both groups was a high school graduated, and most respondents in both groups did not work. The BMI of most respondents in the TB+DM group was 18.5–25, while the TB-DM group was <18.5, so based on the Chi-square test there were significant differences between the two groups (Table 1).

Table 1: Relationship between demographic status and the occurrence of DM in TB patients

Variables	TB with DM				p-value
	Yes		No		
	F	%	F	%	
Gender					0.307
Male	36	34.3	32	30.5	
Female	15	14.3	22	21.0	
Age (years)					0.019
≤30	3	2.9	13	12.4	
31–45	14	13.3	19	18.1	
46–60	26	24.8	15	14.3	
>61	8	7.6	7	6.7	
Education					0.694
Never did school	2	1.9	1	1.0	
Not graduated elementary school	6	5.7	5	4.8	
Graduated elementary school	12	11.4	9	8.6	
Graduated middle school	12	11.4	10	9.5	
Graduated high school	15	14.3	23	21.9	
Graduated university, master degree, doctoral degree	4	3.8	6	5.7	
Work					0.43
Does not work	13	12.4	22	21.0	
Farmer	7	6.7	3	2.9	
Labor	8	7.6	6	5.7	
Trader	6	5.7	4	3.8	
Non-government/ government employee	9	8.6	8	7.6	
Others	8	7.6	11	10.5	
BMI					0.027
<18.5	14	13.3	28	26.7	
18.5–25	28	26.7	22	21.0	
>25	9	8.6	4	3.8	

DM: Diabetes mellitus, TB: Tuberculosis, BMI: Body mass index

Among TB+DM respondents, most suffered DM previous and then become infected by TB. For respondents who suffered TB, the period of TB to the diagnosis of DM is 0–5 years. For respondents who were diagnosed with diabetes first, the period for most respondents until they were diagnosed with TB were 0–5 years. Most respondents in this category are patients. Respondents who were diagnosed with TB in the same year as DM were respondents who did not know that they had been sick with DM before. The diagnosis of DM is made by examining HbA1c, and most respondents in this category are also new patients (Table 2).

TNF-α test to determine protein concentration was carried out by the ELISA sandwich test, and the protein concentration found with a standard curve.

Fig. 1 illustrated the distribution pattern of TNF-α protein concentration found in serum from respondents suffering TB-DM and TB+DM. Mann–Whitney test was performed to determine the difference in the average TNF-α protein concentration in the two groups. Moreover, it was found that there were most significant differences in TNF-α protein concentration serum between the TB-DM group and TB+DM. TB-DM group had averaged of TNF-α protein concentration serum was higher than TB+DM group (Table 3).

Fig. 2 illustrated the distribution of IFN-γ plasma proteins concentration on the TB-DM group and TB+DM group. After the Mann–Whitney test conducted, there are no significant differences in IFN-γ protein

Table 2: Categories of TB+DM response based on diagnosis

Diagnosis	F	%
TB first (n=2)		
Range of years		
0–5 years	2	3.9
6–10 years	0	0.0
11–15 years	0	0.0
>15 years	0	0.0
Categories		
New patient	1	2.0
Relapsed patient	1	2.0
Dropped of medication patient	0	0.0
DM first (n=28)		
Range of years		
0–5 years	17	33.3
6–10 years	7	13.7
11–15 years	1	2.0
>15 years	3	5.9
Categories		
New patient	26	51.0
Relapsed patient	2	3.9
Dropped of medication patient	0	0.0
Diagnosed in the same year (n=21)		
Categories		
New patient	17	33.3
Relapsed patient	1	2.0
Dropped of medication patient	3	5.9

DM: Diabetes mellitus, TB: Tuberculosis

Table 3: Concentrations of TNF-α (pg/mL) and IFN-γ (IU/mL) in TB-DM and TB+DM

	Mean (min-max)	Standard deviation	p-value
TNF-α			
TB-DM	5.2 (0.1–14)	3.486	0.00
TB+DM	2.06 (0.1–7.4)	1.979	
IFN-γ			
TB-DM	1.5 (0.02–7.72)	1.83	0.727
TB+DM	1.3 (0.05–4.45)	1.404	

TNF-α: Tumor necrosis factor-alpha, IFN-γ: Interferon-gamma, DM: Diabetes mellitus, TB: Tuberculosis

concentration between the two groups. However, TB-DM group had averaged of IFN-γ protein concentration was higher than TB+DM group (Table 3).

DISCUSSION

TB still a serious threat to public health. A study conducted by Zin *et al*. revealed that male aged 20–59 years more often infected by TB [6]. Research conducted by Fahmi in TB+DM cases, male also become the dominant sufferers, with most was <30 years old [7]. This study also found that male had more TB, both the TB+DM and TB-DM groups, but did not affect the occurrence of TB+DM. Age range 46–60 years more had susceptible TB+DM; whereas, in the TB-DM group, age 31–45 years had most. This age range difference had affected to the TB+DM condition. Harso *et al*. and Illahi *et al*. mentioned similar results that the age of 45–54 years was more susceptible to had TB+DM, and the age range of 25–34 years had TB-DM [8,9]. In addition, Arlinda indicated similar results [10]. Thus, we proposed that age can be classified as a risk factor of having TB+DM.

For education level categories, most of the respondents were high school graduated but did not affect the occurrence of TB+DM cases. The same result was obtained by Harso *et al*. that the level of education graduated from high school was more dominant in both groups [8]. Most respondents did not have permanent jobs and work also did not affect to TB+DM cases. This discovery is different from what by Fahmi mentioned that most TB patients had jobs [7]. Based on BMI data, we found that most of the respondents had a normal BMI data for both groups, and there is a significant difference in BMI data between the two groups.

DM patients are more susceptible to suffering TB. This study demonstrated that most TB patients had already suffered from diabetes. This discovery is according to a research by Hayashi and Chandramohan who conducted a meta-analysis of 14 studies [11]. Most respondents had a period of 0–5 years from being diagnosed with DM to being diagnosed with TB and are new TB patients.

The results also found that many respondents were diagnosed with TB and DM in the same year. This can happen because the respondent did not know that they had DM. This case instigated by the symptomatic illness lead to patients did not come to the health centers. Moreover, the patients had a low interest to conducted regular health check-up to detect the disease sooner. Acknowledgment of DM disease caused them did not received treatment for blood glucose control. Immunity impaired in DM sufferers made it more susceptible to TB disease [5].

The immune response against M.tb is mediated by cytokines and Th1 cells, which contributes in eliminating microbacterial infection. IFN-γ is the main cytokine that was involved in the immune response against micro bacteria. Its main function is the activation of macrophage, allowing them to exert the microbicidal function. TNF-α has a primordial function to synergize with IFN-γ, stimulating the production of reactive nitrogen intermediates. It will mediate the function of tuberculostatic macrophages, stimulates the migration of immune cells to the location of the infection, and contributes to the formation of granulomas [12].

DM in patients with pulmonary TB causes failure in treatment and worsens the disease compared to pulmonary TB-DM. The rifampicin serum concentration in hyperglycemia patients was found lower also [13]. Researches that have been done about the related between DM and TB showed that DM was an important risk main factor in the developed of TB. Increased risk and severity of TB disease, DM had a significant negative impacted on public health, especially in countries where both conditions were common. According the complexity of the diabetes complications mechanism and the many things involved, the possibility of an immune responded to M.tb infection was affected at many levels [14].

Pulmonary TB disease with diabetes was signed by an increased cytokine responded that indicated the presence of chronic inflammation that underlies type 2 diabetes. This had the potential to increased immune pathology and poor control in pulmonary TB infection [5]. The effector function for bacterial elimination was mediated by macrophages activated by cytokines derived from T lymphocytes, specifically IFN-γ, and TNF-α [12].

The results presented in Fig. 1. was showed that TNF-α concentration in serum was lower in TB with DM group. It was indicated that the responded of the immune body on a group of TB-DM was much better than on a group of TB+DM. Similar results revealed by Cheekatla *et al*., who researched mice [15]. However, a different result was stated by Raposo-García *et al*. that said there was an increased in TNF-α production in M.tb infected blood on patients with DM [16]. A causes by

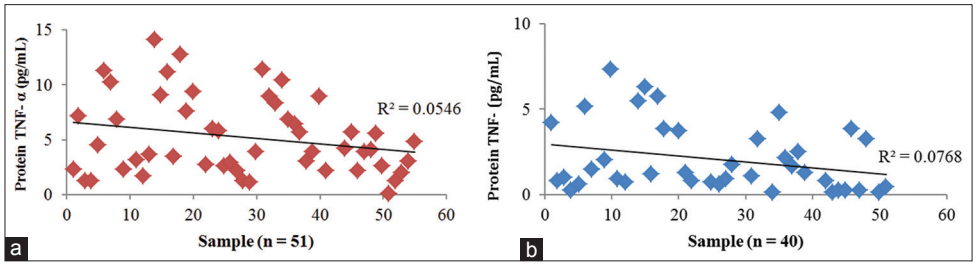

Fig. 1: (a and b) Distribution of tumor necrosis factor-alpha protein concentations in tuberculosis without diabetes mellitus group and TB+DM group

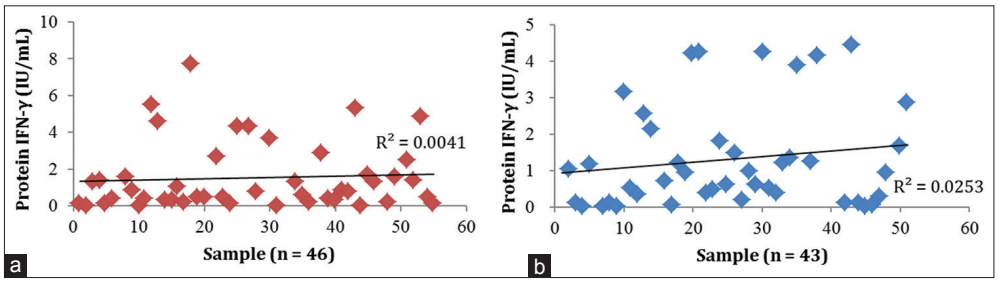

Fig. 2: (a and b) Distribution of the concentration interferon-gamma proteins concentations in tuberculosis without diabetes mellitus group and TB+DM group

different results in this research were the blood sample that was usage from patients with diabetes that incubated with M.tb. The result was also different from research by Lachmandas *et al*. that conducted the research *in vitro* using peripheral blood mononuclear cells from healthy individual [3].

Fig. 2. shows that there were no significant differences in the IFN-γ protein concentration between the two groups, but the average of TB-DM was higher than TB+DM. The same result was also revealed by Kumar *et al*. In the TB-DM group, there was an increased in IFN-γ compared to the TB-DM group [17]. Other research showed a similar condition that there was an impaired of immune response in DM patients with hyperglycemia, as marked by an increase of IFN-γ concentration, and caused the changes in the regulation of cytokine Th1 that was created by the increased of the protein end product [18].

Our result showed a decreased in the immune response in patients with TB+DM. The impaired immune response will compromise the body's defense system to fight TB infection. This might explain the high number of TB disease in DM patients.

CONCLUSION

A declined in the immune response of TB patients with DM was marked by the decreased of TNF-α and IFN-γ protein expression. This decreased in immune responded might led to the DM patients more susceptible to suffered TB.

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AUTHORS' CONTRIBUTIONS

Authors' contributions were as follows: Nelly Marissa wrote the manuscript, Nur Ramadhan and Eka Fitria collect the samples, Sari Hanum and Marlinda measured ELISA and IGRA of markers, Abidah Nur data analysis, and correction.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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