METFORMIN ASSOCIATED INFLAMMATION LEVELS REGULATION IN TYPE 2 DIABETES MELLITUS-TUBERCULOSIS COINFECTION PATIENTS – A CASE REPORT

ABSTRACT

IFN-γ elevation is one of the indicators of successful treatment in active tuberculosis (TB) infection due to macrophage and Th-1 activation in inducing autophagy process. However, IL-10 also inhibits interferon-mediated mycobactericid activities by blocking IFN-γ signaling pathways in autophagy. Therefore, ratio IFN-γ / IL-10 has to be greater than 1 (>1) then IFN-γ remains has anti-mycobacterium. Metformin (MET) is a potent combination drug to elevate anti-TB efficacy and able to regulate inflammation.

In this study, an observational clinical study was done in diabetes mellitus (DM)-TB co-infection outpatients at Surabaya Paru Hospital. This study evaluated how MET therapy affected inflammation. MET was used at least 2 months, accompanying with insulin and anti-TB and as comparison to non MET group.

The result in this study MET increased both pro-inflammatory and anti-inflammatory cytokines, thus MET may consider as adjunct therapy in DM-TB coinfection patients due to its ability in Th-1 and Th-2 immuno-regulating response, especially to enhance IFN-γ and to reduce insulin associated IL-10 upregulation.

Keywords: type 2 diabetes mellitus-tuberculosis co-infection; metformin; ratio interferon gamma and interleukin-10

1. INTRODUCTION

Mycobacterium tuberculosis (M. tuberculosis) infection or known by TB infection is a leading cause of global morbidity and mortality thus, requiring long-term therapy[1,2].

There are five phases of M.tuberculosis infection and divided into two main phase. Firstly

is invasion phase (phase 1-2) and secondly is immunological phase, this phase is happened due to the immunology response during interaction of M. tuberculosis and host (phases 3-5) [3]. Invasion phase occurs when M. tuberculosis reaches the pulmonary alveoli and becomes colony in the lung with its ability to avoid the phagocytosis. In phase 2, M.tuberculosis multiplies in immature nonactivated macrophages to form a lesion called tubercle. In invasion phase, anti TB works well to eliminate M. tuberculosis. However, the efficacy of anti TB reduce in immunology phase, which the host body starts limit M. tuberculosis by developing caseous necrosis as immune response against tuberculin-like antigens released by M.tuberculosis in phase 3, then becomes liquefaction phase in phase 4 to 5 to limited tuberculosis extracellular multiplication [3,4]. The aimed of anti-TB therapy is curing patients, preventing death, preventing recurrence, cutting off transmission chains and preventing germ resistance by eradication of M.tuberculosis. The effectiveness of rifampicin, isoniazid, pyrazinamide and ethambutol is influenced by host immune response[5,6].Therefore, new approach is needed to enhance anti TB efficacy during immunology phase. One of offered suggestion was compiled MET during intensive phase of anti-TB therapy.

Metformin hydrochloride (MET), biguanide, use in type 2 diabetes mellitus by 1) inhibiting the production of hepatic glucose; 2) reducing intestinal glucose absorption; and 3) improving glucose uptake and utilization [5,7,8]. MET is known affecting inflammation mediators, both pro-inflammation, such IFN-γ, IL-β and also anti-inflammation such IL-10 [8–10]. Interferon (IFN)-γ is a potent cytokine that indicates antimicrobial effect and also modulates the production or activities of several cytokines and chemokines [11–13]. IFN-γ activates macrophages and dendritic cells to perform autophagy to M. Tuberculosis, and diminished of IFN-γ relates to anti-tuberculosis therapy failure[14–18].

2. MATERIALS AND METHODS

1.1 Study Design

In this study, an observational clinical study was done in diabetes mellitus (DM)-TB co-infection outpatients at Surabaya Paru Hospital. It involved two groups, MET group as observation group, and non MET group. The MET group was receiving MET therapy with doses from 1000 mg to 1500 mg along with insulin and anti-TB during the intensive periods. The enrolled patients criterias: 1) patient DM with a new case of TB co-infection, who were given insulin and anti-TB regimens; 2) positive sputum smear; 3) Patient's age was 25 to 60 years old; 4) has normal liver function and renal function; 5) not in hypoxia condition, presenting by peripheral oxygen saturation level must be higher than 92%.

The levels of IFN-γ and IL-10 was measured before and after this observation period and as a clinical result, we also evaluated the smear reversion in DM-TB coinfection patients in both groups.

2.2 Diagnosis and Management Therapy

The diagnosis of TB was established by 1) clinical symptoms and signs of TB, such: chronic productive cough, unintentional weight loss; 2) positive sputum smear of acid-fast bacilli (AFB) by microscopic Ziehl-Neelsen-stained sputum slides; and 3) chest radiographs with suggestive features of TB. The diagnosis of DM was established by fasting blood sugar > 120mg/dL; HbA1c > 7%.

Patients diagnosed with TB were registered and treated with anti-TB for 6 months in accordance to WHO guidelines[19,20]. Insulin use for achieving good glycaemic control in the patients in this study. These following drugs were used: MET (Metformin$^{(R)}$), insulin (Humulin$^{(R)}$), rifampicin (RIF), isoniazid (INH), pyrazinamide (PYR), ethambutol (ETH). MET were given 1000 – 1500 mg in the divided daily dose for at least two months or during

67. the intensive phase of anti-TB therapy.

2.3 Acid Fast Bacilli Smears (AFB) Smears

68. Sputum smears were examined two times : 1) before treatment in order to diagnose and
69. 2) after the intensive phase of anti-TB treatment in order to do evaluation.

2.4 Cells Culture and ELISA

70. **Cells and ELISA. Cells.** PBMC was obtained from patients' whole blood and $1x10^6$
71. were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 0,1μg
72. mantoux and 0,7 μg penicilline. Supernatant were harvested after 72 hours and prepare for
73. ELISA methods in order to measure the levels of IFN-γ and IL-10. **ELISA.** IFN-γ (RnD
74. DIF50) and IL-10 (RnD P113058) were used as measurement kits.

3. RESULT

3.1 Characteristic of patients

75. This study's ethical clearance was approved by ethical committee of Surabaya Paru
76. Hospital with no. 09.01/KERS/102.6/2016 and written informed consent obtained from all
77. participants after information for consent was given by the investigators. During this study
78. period, there were 476 cases of new TB infection and 156 cases (~30%) of that were type
79. 2 DM-TB co-infection. 42 patients were eligible participated in this observational studies.
80. All the basic conditions in both groups were homogeneous ($p > 0,05$) (as seen in table 1).
81.
82. In order to prevent MET associated lacto acidosis (MALA) during MET therapy in this
83. study, all patients has been determined as mention at enrolled patients criterias (data was
84. as seen in table 1). Moreover, there was no incidence of lactic acidosis event during this
85. observation period[21].

During observation weeks (intensive phase of anti-TB therapy), we also obtained fasting blood sugar (FBS) levels periodically and the FBS levels were also similar in both groups (as seen in Figure 1). This data show that IFN-γ and IL-10 elevation in this study might be not directly influenced by hyperglycemia condition.

For sputum smears, as seen in Table 2 shows that prior to the intensive phase of anti-TB therapy none of the subjects were having negative AFB in both groups. The highest number of AFB count (+3) in MET group was 40.9% and in non MET group was 35%. After 2 months MET therapy accompanying with insulin and anti-TB regimens, all patients in MET group were AFB reversion (negative smears result), while only 75% of non MET group had AFB reversion. Using the Fisher's exact test, results of different test $p = 0.046$ ($p<0.005$), which means there is a significantly difference of AFB smears reversion between the MET group and the non MET group.

3.2 IFN-γ, IL-10 and ratio IFN-γ, IL-10

3.2.1 IFN-γ. IFN-γ is activated by Th1 and NK Cells to induce macrophage and dendritic cell activation thus provide protection against TB infection[22,23]. Increased of IFN-γ in chronic TB infection is a cellular immune response. Currently, IFN-γ release assay (IGRA) is used as one of the tools of diagnosis of latent TB infection and IFN-γ elevation is one of the indicators of successful treatment in active TB infection[24].

Using *Wilcoxon-Mann Whitney,* nonparametric statistical test, figure 2 shows that IFN-γ level before treatment between MET group and non MET group were alike ($p>0,005$), thus it shows that patients in both groups, before treatment, were in the similar stage of IFN-γ. The differences before and after observation period was

significant in MET group ($p<0,005$) while in non MET group was not. Referring to negative AFB in MET group after 2 months intensive therapy (as seen in figure 2), it supports that IFN-γ has effect as mycobactericid.

3.2.2 IL-10. . IL-10 has ability to inhibit the Th-1pro-inflammation cytokines, including IFN-γ[23–25]. Using *Wilcoxon-Mann Whitney,* nonparametric statistical test, figure 3 shows that IL-10 level before treatment between MET group and non MET group were alike ($p>0,005$), thus it shows that patients in both groups, before treatment, were in the similar stage of IL-10. Although IL-10 level was increased, the differences before and after observation period was not significant between MET and nongroup ($p>0,005$).

3.2.3 Ratio IFN-γ / IL-10. Ratio IFN-γ / IL-10 shows that immune processes inside the host were more dominated by pro-inflammatory or anti-inflammatory cytokines after intensive phase of anti-TB with or without MET therapy. Whenever the IFN-γ / IL-10 ratio is greater than 1 (>1), thus the host's immunity defense system was dominated by pro-inflammation condition[26].

Using *Wilcoxon-Mann Whitney,* table 3 shows that ratio IFN-γ / IL-10 were not significant difference before and after the intensive phase of anti-TB therapy between MET and non MET group ($p>0,005$). However, IFN-γ / IL-10 ratio difference variation after MET combined anti-TB and insulin was narrower than non MET group.

4. DISCUSSION

IFN-γ is the chief cytokine involved in the protective immune response against mycobacterial infection[11,27,28]. The main function of IFN-γ is macrophage and dendritic cells activation, thus in this study autophagy marker was also high[21] and it

referred to its mycobactericid functions. Predominantly IFN-γ is also contributed to less severe forms of pulmonary TB[23]. Moreover, IFN-γ also enhances the antigen pre sentation through the induction of the expression of molecules from the major histocompatibility complex (MHC) class I and class II and promoting the differentiation of CD4 T lymphocytes to the Th1 subpopulation [11,22]. Furthermore as conclusion in this study MET associated to inflammation regulatory in DM-TB coinfection patients. However, IFN-γ relates to CD8 T-lymphocytes or cytotoxic T-cells also contributes to lung tissue damaged, thus IFN-γ activity needs to be controlled[22,23].

IL-10 a major anti-inflammatory cytokines plays important role in metabolic disorder such diabetes due its affect to insulin sensitivity[29]. IL-10 is produced by macrophages and Th-2 during *M. tuberculosis* infection It suppresses macrophage function and inhibits pro-inflammation cytokines such IFN-γ, TNF-σ and IL-1β. The increase in IL-10 levels appears to support the mycobacterial survival in the host[23] due to the inhibition of autophagy targeting signals through IL-10 activated SOCS3, and then, SOCS3 inhibits the Janus kinase-2 (Jak2)/signal transducer and activator of transcription (Stat) pathway in activating macrophage autophagy[27,28]. In this study, the increasing of IL-10 may happen not only due to macrophage related Th-2 activation but also due to insulin attenuated anti-inflammation regulatory[29–32].

Based on this result, MET therapy may consider as new strategy in enhancing anti TB efficacy due to its two main ability : 1) MET controlled IL-10 secretion thus alter host immune response against TB infection; and; 2) MET also affects to insulin sensitivity thus enhanced insulin therapy.

Regulating pro-inflammatory and anti-inflammatory cytokines is a critical role in the

immunity and progression of inflammation. Knowing the use of "old" drug, MET, for new strategy in conquering TB was the purpose of this case-study. As conclusion in this study, MET increased both pro-inflammatory and anti-inflammatory cytokines, thus MET may consider as adjunct therapy in type 2 DM-TB coinfection patients due to its ability in Th-1 and Th-2 immuno-regulating response. However, the further study requires in knowing MET attenuated host sensitivity against *M. tuberculosis* infection in a larger number of DM-TB coinfection patients.

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CONFLICT OF INTEREST

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