

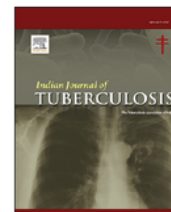
Metformin associated inflammation levels regulation in type 2 diabetes mellitus- tuberculosis coinfection patients e A case report

by Bernadette Dian Novita

FILE	1-METFORMIN_ASSOCIATED_.PDF (379.88K)		
TIME SUBMITTED	15-FEB-2019 03:52PM (UTC+0700)	WORD COUNT	3799
SUBMISSION ID	1078613734	CHARACTER COUNT	20244

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: <http://www.journals.elsevier.com/indian-journal-of-tuberculosis/>

Original article

Metformin associated inflammation levels regulation in type 2 diabetes mellitus-tuberculosis coinfection patients – A case report

Bernadette Dian Novita ^{a,*}, Endang Isbandiati Soediono ^a, Jusak Nugraha ^b^a Department of Pharmacology, Faculty of Medicine, Widya Mandala Catholic University, Surabaya, Indonesia^b Department of Clinical Pathology, Faculty of Medicine, University of Airlangga/ Dr. Soetomo Hospital, Surabaya, Indonesia

ARTICLE INFO

Article history:

Received 27 August 2018

Accepted 31 August 2018

Available online 11 September 2018

1

Keywords:

Type 2 diabetes mellitus-tuberculosis co-infection

Metformin

Ratio interferon gamma and interleukin-10

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 mycobactericidal 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.

© 2018 Tuberculosis Association of India. Published by Elsevier B.V. All rights reserved. 12

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

* Corresponding author.

E-mail address: novita@ukwms.ac.id (B.D. Novita).<https://doi.org/10.1016/j.ijtb.2018.08.006>

0019-5707/© 2018 Tuberculosis Association of India. Published by Elsevier B.V. All rights reserved.

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 4–5 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 immunity 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

2.1. 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 25–60 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) 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 (FBS) >126 mg/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 glycemic control in

the patients in this study. These following drugs were used: MET (Metformin[®]), insulin (Humulin[®]), 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 the intensive phase of anti-TB therapy.

2.3. Acid fast bacilli smears (AFB) smears

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

2.4. Cells culture and ELISA

Cells and ELISA. Cells. MC was obtained from patients' whole blood and 1×10^6 were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 0.1 μ g mantoux and 0.7 μ g penicilline. Supernatant were harvested after 72 hours and prepare for ELISA methods in order to measure the levels of IFN- γ and IL-10. ELISA. IFN- γ (RnD DIF50) and IL-10 (RnD P113058) were used as measurement kits.

3. Result

3.1. Characteristic of patients

This study's ethical clearance was approved by ethical committee of Surabaya Paru Hospital with no. 09.01/KERS/102.6/2016 and written informed consent obtained from all participants after information for consent was given by the investigators. During this study period, there were 47 cases of new TB infection and 156 cases (~30%) of that were type 2 DM-TB co-infection. 42 patients were eligible participated in this observational studies. All the basic conditions in both groups were homogeneous ($p > 0.05$) (as seen in Table 1).

In order to prevent MET associated lacto acidosis (MALA) during MET therapy in this study, all patients has been determined as mention at enrolled patients criterias (data was as seen in Table 1). Moreover, there was no incidence of lactic acidosis event during this observation period.²¹

During observation weeks (intensive phase of anti-TB therapy), we also obtained FBS levels periodically and the

Table 1 – Characteristic of type 2 DM-TB coinfection during observation period of study.

Parameters	MET group	Non MET group	p (difference)
Ages (years old)	44.59 \pm 8.64	48.40 \pm 8.17	0.863
HbA1c (%)	8.82 \pm 1.91	9.52 \pm 2.02	0.379
Oxygen saturation (SpO ₂) (%)	98.06 \pm 0.73	97.47 \pm 0.83	0.308
BUN (mg/dL)	0.95 \pm 0.16	0.93 \pm 0.13	0.980
Creatinine serum (U/L)	23.92 \pm 11.92	27.3 \pm 12.01	0.103
SGOT (U/L)	17.63 \pm 6.16	14.44 \pm 6.48	0.354
SGPT (U/L)	19.22 \pm 8.73	16.09 \pm 7.56	0.509
Participants characteristic condition before observation periods. HbA1c was measured after 2 months observation period.			

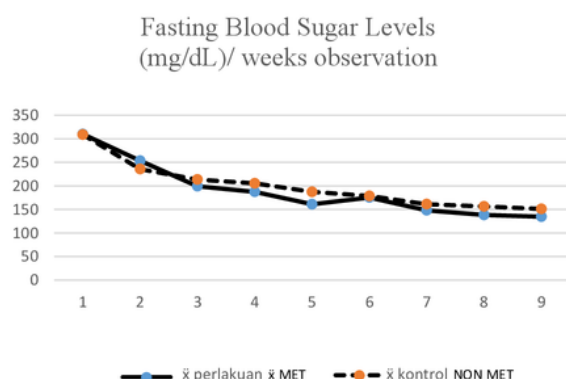


Fig. 1 – Fasting blood sugar levels of type 2 DM-TB coinfection during observation period of study.

FBS levels were also similar in both groups (as seen in Fig. 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, Fig. 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

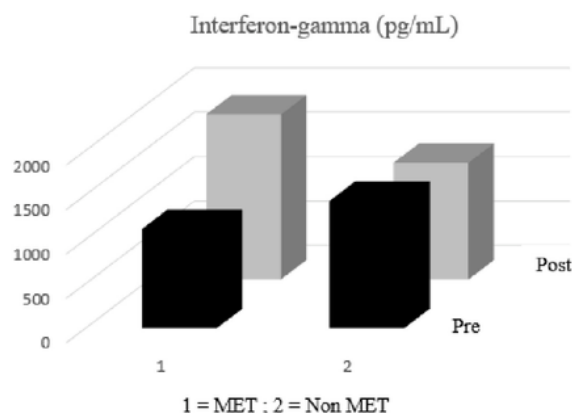


Fig. 2 – Interferon (IFN)- γ level of type 2 DM-TB patient before and after observation period. Difference of interferon gamma level pre and post observation in MET group was significantly different from non MET group.

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 Fig. 2), it supports that IFN- γ has effect as mycobactericid.

3.2.2. IL-10

IL-10 has ability to inhibit the Th1-pro-inflammation cytokines, including IFN- γ .^{23–25} Using Wilcoxon-Mann Whitney, nonparametric statistical test, Fig. 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 ($p > 0.005$).

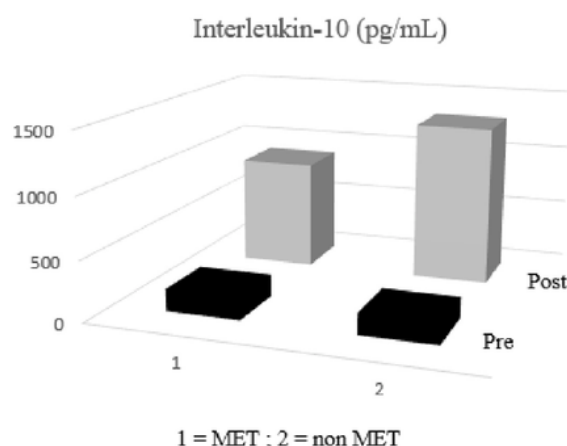


Fig. 3 – Interleukin (IL)-10 level of type 2 DM-TB patient before and after observation period. Difference of interleukin-10 level pre and post observation in MET group was not significantly different from non MET group.

Table 2 – Acid Fast Bacilli Sputum Smears result of type 2 DM-TB patients before and after observation period.

AFB result	MET group (N %)		Non MET group (N %)	
	Before	After	Before	After
Negative	0 (0%)	22 (100%)	0 (0%)	15 (75%)
Scanty/ + 1	9 (40.9%)	0 (0%)	6 (30%)	5 (25%)
+2	4 (18.2%)	0 (0%)	7 (35%)	0 (0%)
+3	9 (40.9%)	0 (0%)	7 (35%)	0 (0%)
Total	22 (100%)	22 (100%)	20 (100%)	20 (100%)

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 high²¹ 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 presentation 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- α and IL-1 β . The increase in IL-10 levels appears to support the mycobacterial survival in the host²³ 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}

Table 3 – Ratio Interferon (IFN)- γ /Interleukin (IL)-10 level of type 2 DM-TB patient before and after observation period.

Ratio IFN- γ /IL-10	MET group	Non MET group	Between groups differences
	$\bar{x} \pm SD$	$\bar{x} \pm SD$	
Before	8.99 \pm 5.67	11.65 \pm 6.17	0.247
After	2.18 \pm 0.53	6.74 \pm 4.35	0.212
Delta	0.904 \pm 0.808	2.68 \pm 6.17	0.433

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.

Conflicts of interest

All participants in this study were voluntary involved and funding was written in the acknowledgement.

Acknowledgement

The authors would like to thank almighty God for helping us complete this work successfully. We thank Sri Hariastuti and team (Clinical Pathology, Diagnostic Center Dr. Soetomo Hospital, Surabaya, Indonesia) for her technical assistance. We thank Wuryani, dr., Sp.PD; Kusdiantoro, dr., Sp.P and Indrayana, dr., Sp.P as our clinical mentors in Surabaya Paru Hospital. We also thank Prof. Dr., Moh. Amin, dr., Sp.P(K); Prof. Dr. Ni Made Mertaniasih, dr., MS., Sp.MK(K); Prof. Dr. Agung Pranoto, dr., M.Kes., Sp.PD, K-EMD, FINASIM; Dr. Hari Basuki, dr., MS., and Prof. Dr. Chiou-Feng Lin (Department of Microbiology and Immunology, School of Medicine, Taipei Medical University, Taipei, Taiwan) for the kindly discussion.

REFERENCES

- WHO. Collaborative framework for care and control of tuberculosis and diabetes. *World Heal Organ*. 2011;314(5805):2, 978 92 4 150225 2.
- Kementerian Kesehatan Republik Indonesia. *Pedoman Nasional Pengendalian Tuberkulosis*. 2014:24–25.
- Caire-Brändli I, Papadopoulos A, Malaga W, et al. Reversible lipid accumulation and associated division arrest of *Mycobacterium avium* in lipoprotein-induced foamy macrophages may resemble key events during latency and reactivation of tuberculosis. *Infect Immun*. 2014;82(2):476–490. <https://doi.org/10.1128/IAI.01196-13>.
- Prezzemolo T, Guggino G, La Manna MP, Di Liberto D, Dieli F, Caccamo N. Functional signatures of human CD4 and CD8 T cell responses to *Mycobacterium tuberculosis*. *Front Immunol*. 2014;5(8):180. <https://doi.org/10.3389/fimmu.2014.00180>.
- Brunton L, Chapner B, Knollmann B. In: Brunton L, Chapner B, eds. *The Pharmacological Basis of Therapeutics-Goodman & Gilman*. 12th ed. San Diego, California: Mc Graw Hill Medical; 2011.

6. V Almeida D. Revisiting anti-tuberculosis activity of pyrazinamide in mice. *Mycobact Dis*. 2014;04(02):2–7. <https://doi.org/10.4172/2161-1068.1000145>.
7. Katzung BG, Mastres SB, Trevor AJ. *Basic & Clinical Pharmacology*. 14th ed. Singapore: Mc Graw Hill Education (Asia); 2018.
8. Marupuru S, Senapati P, Pathadka S, Miraj SS, Unnikrishnan MK, Manu MK. Protective effect of metformin against tuberculosis infections in diabetic patients: an observational study of south Indian tertiary healthcare facility. *Braz J Infect Dis*. 2017;21(3):312–316. <https://doi.org/10.1016/j.bjid.2017.01.001>.
9. Dallaglio K, Bruno A, Cantelmo AR, et al. Paradoxical effects of metformin on endothelial cells and angiogenesis. *Carcinogenesis*. 2014;35(5):1055–1066. <https://doi.org/10.1093/carcin/bgu001>.
10. Coyle C, Cafferty FH, Vale C, Langley RE. Metformin as an adjuvant treatment for cancer: a systematic review and meta-analysis. *Ann Oncol*. 2016;27(12):2184–2195. <https://doi.org/10.1093/annonc/mdw410>.
11. Chee CBE, KhinMar KW, Gan SH, et al. Tuberculosis treatment effect on T-cell interferon-gamma responses to *Mycobacterium tuberculosis*-specific antigens. *Eur Respir J*. 2010;36(2):355–361. <https://doi.org/10.1183/09031936.00151309>.
12. Cuccu B, Freer G, Genovesi A, Garzelli C, Rindi L. Identification of a human immunodominant T-cell epitope of mycobacterium tuberculosis antigen PPE44. *BMC Microbiol*. 2011;11(1):167. <https://doi.org/10.1186/1471-2180-11-167>.
13. Abbas AK, Lichtman AH. *Cellular and Molecular Immunology*. 7th ed. Elsevier Inc.; 2012. <https://doi.org/10.1016/B978-0-7020-3369-8.00001-X>.
14. Lemos MP, Rhee KY, McKinney JD. Expression of the leptin receptor outside of bone marrow-derived cells regulates tuberculosis control and lung macrophage MHC expression. *J Immunol*. 2011;187(7):3776–3784. <https://doi.org/10.4049/jimmunol.1003226>.
15. Kresno SB. *Imunologi: Diagnosis Dan Prosedur Laboratorium*. V. Badan Penerbit Fakultas Kedokteran Universitas Indonesia; 2013.
16. Bertholet S, Horne DJ, Laughlin EM, et al. Effect of chemotherapy on whole-blood cytokine responses to *Mycobacterium tuberculosis* antigens in a small cohort of patients with pulmonary tuberculosis. *Clin Vacc Immunol*. 2011;18(8):1378–1386. <https://doi.org/10.1128/CI.05037-11>.
17. Sakai S, Kauffman KD, Sallin MA, et al. CD4 T cell-derived IFN- γ plays a minimal role in control of pulmonary *Mycobacterium tuberculosis* infection and must be actively repressed by PD-1 to prevent lethal disease. *PLOS Pathog*. 2016;12(5):e1005667. <https://doi.org/10.1371/journal.ppat.1005667>. Fortune SM, ed.
18. Herzmann C, Ernst M, Ehlers S, et al. Increased frequencies of pulmonary regulatory T-cells in latent *Mycobacterium tuberculosis* infection. *Eur Respir J*. 2012;40(6):1450–1457. <https://doi.org/10.1183/09031936.00214611>.
19. Menzies D, Sterling TR. Treatment of *Mycobacterium tuberculosis* infection: time to get a move on? *Ann Intern Med*. 2014;161(6):449. <https://doi.org/10.7326/M14-1719>.
20. van Deun A, Monedero I, Rieder HL, et al. In: Caminero J, ed. *Guidelines for Clinical and Operational Management of Drug-Resistant Tuberculosis*. 2013.
21. Novita BD, Pranoto A, Wuryani, Soediono EI, Mertaniasih NM. A case risk-study of lactic acidosis risk in metformin use in type 2 diabetes mellitus tuberculosis co-infection patients. *Indian J Tubercul*. 2017. <https://doi.org/10.1016/j.ijtb.2017.05.008>.
22. Abbas AK, Lichtman A. In: Abbas AK, Lichtman A, Pillai S, eds. *Cellular and Molecular Immunology*. 7th ed. Philadelphia, PA: Saunders; 2012.
23. Cavalcanti YVN, Brelaz MCA, Neves JKDAL, Ferraz JC, Pereira VRA. Role of TNF-alpha, IFN-gamma, and IL-10 in the development of pulmonary tuberculosis. *Pulm Med*. 2012. <https://doi.org/10.1155/2012/745483>.
24. Matsushita I, Le Hang NT, Hong LT, et al. Dynamics of immune parameters during the treatment of active tuberculosis showing negative interferon gamma response at the time of diagnosis. *Int J Infect Dis*. 2015;40:39–44. <https://doi.org/10.1016/j.ijid.2015.09.021>.
25. Singhal J, Agrawal N, Vashishta M, et al. Suppression of dendritic cell-mediated responses by genes in calcium and cysteine protease pathways during *Mycobacterium tuberculosis* infection. *J Biol Chem*. 2012;287(14):11108–11121. <https://doi.org/10.1074/jbc.M111.300319>.
26. Alam K, Ghousunnissa S, Nair S, Valluri VL, Mukhopadhyay S. Glutathione-redox balance regulates c-rel-driven IL-12 production in macrophages: possible implications in antituberculosis immunotherapy. *J Immunol*. 2010;184(6):2918–2929. <https://doi.org/10.4049/jimmunol.0900439>.
27. Lin C, Chien S, Chen C, et al. IFN- γ induces mimic extracellular trap. *J Interf Cytokine Res*. 2015;36(2):1–13. <https://doi.org/10.1089/jir.2015.0011>.
28. Lin C, Lin C, Lee K, et al. Escape from IFN- γ -dependent immunosurveillance in tumorigenesis. *J Biomed Sci*. 2017;24(10):1–9. <https://doi.org/10.1186/s12929-017-0317-0>.
29. Barry JC, Shakibakho S, Durrer C, et al. Hyporesponsiveness to the anti-inflammatory action of interleukin-10 in type 2 diabetes. *Sci Rep*. 2016;6(1):21244. <https://doi.org/10.1038/srep21244>.
30. Dobrian AD, Ma Q, Lindsay JW, et al. Dipeptidyl peptidase IV inhibitor sitagliptin reduces local inflammation in adipose tissue and in pancreatic islets of obese mice. *Am J Physiol Endocrinol Metab*. 2011;300(2):E410–E421. <https://doi.org/10.1152/ajpendo.00463.2010>.
31. Clark I, Atwood C, Bowen R, Paz-filho G, Vissel B. Tumor necrosis factor-induced cerebral insulin resistance in Alzheimer's disease links numerous treatment rationales. *Pharmacol Rev*. 2012;64(4):1004–1026. <https://doi.org/10.1124/pr.112.005850>.
32. Hartman ME, O'Connor JC, Godbout JP, Minor KD, Mazzocco VR, Freund GG. Insulin receptor substrate-2-dependent interleukin-4 signaling in macrophages is impaired in two models of type 2 diabetes mellitus. *J Biol Chem*. 2004;279(27):28045–28050. <https://doi.org/10.1074/jbc.M404368200>.

Metformin associated inflammation levels regulation in type 2 diabetes mellitus-tuberculosis coinfection patients e A case report

ORIGINALITY REPORT

% 12	% 9	% 6	% 4
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	repository.unair.ac.id Internet Source	% 4
2	www.ncbi.nlm.nih.gov Internet Source	% 2
3	Ogbera, Anthonia Okeoghene, Anil Kapur, Hussein Abdur-Razzaq, Anthony D Harries, Kaushik Ramaiya, Olufunmilayo Adeleye, and Sonny Kuku. "Clinical profile of diabetes mellitus in tuberculosis", BMJ Open Diabetes Research & Care, 2015. Publication	% 2
4	Whetzel, C.A.. "Disruption in Th1/Th2 immune response in young adult smokers", Addictive Behaviors, 200701 Publication	% 1
5	Submitted to SVKM International School Student Paper	% 1

6	Internet Source	%1
7	Submitted to University of Basrah Student Paper	<%1
8	www.genego.com Internet Source	<%1
9	www.wjgnet.com Internet Source	<%1
10	centaur.reading.ac.uk Internet Source	<%1
11	journals.plos.org Internet Source	<%1
12	ir.nctu.edu.tw Internet Source	<%1

EXCLUDE QUOTES ON
EXCLUDE ON
BIBLIOGRAPHY

EXCLUDE MATCHES < 10
WORDS