

Hyperglycemia Increased The Expression of TLR2 mRNA and Coronin-1A Level in Lung Mice which were infected by Mycobacterium Tuberculosis

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Abstract: The present study was conducted to evaluate the expression of TLR2 mRNA and Coronin-1A level in mice which were infected with *Mycobacterium tuberculosis* (M.tb) and hyperglycemia. A total of 24 mice were divided into 2 groups and all were infected with M.tb, then control group were injected of 5 µl a sodium citrate buffer intraperitoneally and treatment group were injected 150 mg/kg bw streptozotocin (STZ) dissolved in 5 µl sodium citrate buffer intraperitoneally to make hyperglycemia. Lung tissue harvesting conducted for RT-PCR examination for expression of TLR2 mRNA and Coronin-1A level at 3 and 5 week. In this study found the expression of TLR2 mRNA and Coronin-1A level in mice infected with M.tb and hyperglycemia which were examined in the fifth week were significant higher than others.

Keywords: TLR2 mRNA, Coronin-1A, *Mycobacterium tuberculosis*

1. Introduction

TLR2 and TLR6 found to play a role in stimulating production of IL-1β. TLR2 also contribute for IL-12 secretion by macrophages.¹ In TLR2-deficient mice showed granuloma formation is not perfect, when it infected with a high dose of *Mycobacterium tuberculosis* (M.tb) there is susceptibility increase compared to normal mice. TLR2-deficient mice also showed impaired in control of M.tb chronic infection.² In Type 1 diabetes showed increase expression of TLR2 and TLR4 mRNA on peripheral blood monocytes compared to healthy. Final target TLR such as nuclear factor κB, myeloid differentiation factor 88 (MYD 88), Trif, and phosphorylated IL-1 receptor-associated kinase was significantly increased in type 1 diabetes. TLR2 and TLR4 expression is significantly associated with glycosylated hemoglobin.³ In type 2 diabetes with tuberculosis, type 1 (IL-12, IL-2 and IFN-γ) and natural (IL-1β, IL-6, TNFα, IL-8, and granulocyte monocyte-CSF) cytokines are consistently increased in tuberculosis with chronic hyperglycemia compared with tuberculosis patients without diabetes.⁴

TLR is important in killing process of mycobacterium by macrophages through induction of intracellular antimicrobial peptide cathelicidin. TLR polymorphism was significantly associated with tuberculosis in humans. In protein analysis, composition of phagosome contain M.tb indicate the presence of very strong proprietary protein plays a role in M.tb survival, named TACO (tryptophan-aspartate containing coat protein) which is now referred as Coronin-1A. In both patients with active and latent tuberculosis showed increased levels of Coronin-1A compared to healthy.⁵

2. Material and Methods

A total of 24 Balb/c mice, male, 20-25 gr, 8 weeks old were randomly placed in four cages and adapted for 2 weeks. Group I and II consisted of each 12 mice which were placed in cages 1, 2, 3 and 4. In the first day after adaptation, both of groups were infected by 60 µl (with 10⁵ CFU/ml of concentration) M.tb H37Rv intranasally. In group of treatment also injected with 150 mg/kg bw STZ dissolved in 5 µM sodium citrate buffer (pH 4.5) after overnight fasting, while in group of control only received intraperitoneal injection of sodium citrate buffer. Two weeks after intervention, blood sugar level were measured, state diabetes mellitus if the fasting glucose level > 200 mg / dl and unchanged until 5th week. On the third and fifth week after initial treatment, there were 12 mice of each group terminated. The pulmonary organs were prepared divided in two part. First part were submerged in RNA later (Qiagen) for examining TLR2 mRNA and other part were submerged in PBS for examining Coronin-1A with ELISA methods (due to BT-lab kit procedure).

3. Results

a. The expression of TLR2 mRNA

Mean relative of TLR2 mRNA expression in K1: groups of tuberculosis (+) without hyperglycemia (STZ-) in third week is 0.003376. In K2: groups of tuberculosis (+) without hyperglycemia (STZ -) in fifth week is 0.003247. In P1: groups of tuberculosis (+) with hyperglycemia (STZ +) in third week is 0.003625. In P2: groups of tuberculosis (+) with hyperglycemia (STZ +) in fifth week 0.004972. Based on One Way ANOVA found there were significant differences in TLR2 mRNA expression between groups (p = 0.013). In post hoc test with LSD found there were significant difference (p < 0.05) in TLR2 mRNA expression between groups P2 vs P1; P2 vs K2; and P2 vs K1. While there were no significant difference in TLR2 mRNA expression between groups K1 vs K2 (p = 0.807); between

K1 vs P1 ($p = 0.639$) and also between P1 vs K2 ($p = 0.478$). The curve of mean relative TLR2 mRNA expression in group of study were showed in Fig.1

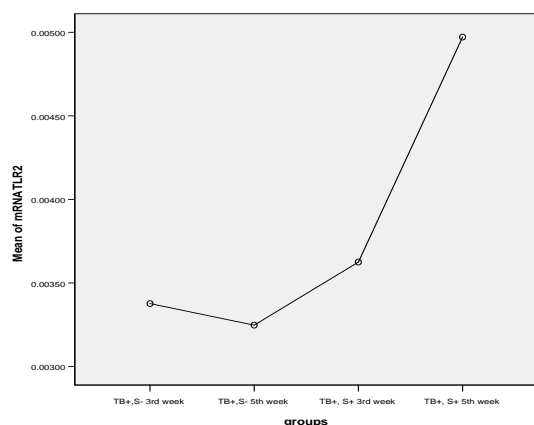


Figure 1: Mean relative curve of TLR2 mRNA expression in groups of study

b. Coronin-1A level

Mean of Coronin-1A level in groups P1 (210,2) pg/ml; P2 (350,4) pg/ml; K1 (174,2) pg/ml and K2 (175,6) pg/ml. Normality test with Shapiro-Wilk result in normal distribution ($p > 0.05$). Homogeneity test with Levene's test showed homogen data ($p = 0.391$). Based on One Way ANOVA test found there were significant differences between groups in Coronin-1A levels ($p = 0.00$). Post hoc test with LSD also found there were significant difference ($p = 0.00$) in Coronin-1A level between P2 vs K2; P2 vs K1; and P2 vs P1). While there were no significant difference in Coronin-1A level between groups K1 vs K2 ($p = 0.693$); between K1 vs P1 ($p = 0.246$) and also between P1 vs K2 ($p = 0.265$). Mean of Coronin-1A level (pg/ml) in all group of treatment showed in Fig.2

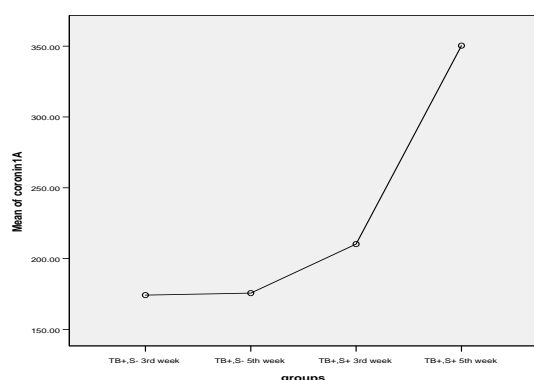


Figure 2: Coronin-1A levels(pg/ml) curve in all groups of treatment

4. Discussion

a. TLR2 mRNA expression

In vivo study with MyD88 deficient mice compared to TLR deficient mice showed MyD88 deficient mice were highly susceptible to respiratory infections by M.tb than TLR-deficient mice. Susceptibility of TLR2-deficient mice to M.tb varies from several different studies, whereas mice with TLR4 deficiency did not show high susceptibility to M.tb infection. Report mentions that TLR9 deficient mice

were susceptible to M.tb infection and more vulnerable if its deficient in both TLR2 and TLR9. These findings indicated that TLRs were important in first binding to mycobacteria. The latest report said that mice deficient TLR2 / TLR4 / TLR9 showed phenotype abnormalities milder than MyD88-deficient mice. Further study was needed to know if any other molecules besides TLRs participate in MyD88 signalling. TLR or MyD88 is an independent component of natural immunity involved in induction of adaptive immune response during M.tb infection.⁶ In experimental studies with rats given STZ to make diabetic and infected M.tb intravenously, showed decrease in IL-12 and IFN- γ level in lung, liver and lymph compared to control mice that were not diabetic. There was also a decrease in expression of inducible nitric oxide synthase (iNOS) compared to control mice that were not diabetic. In mice diabetes appears increase number of M.tb in lung, liver and lymph.⁷

In patients with type 1 diabetes showed an increase expression of TLR2 and TLR4 mRNA on peripheral blood monocytes surface compared to healthy individuals. The final target TLR such as nuclear factor κ B, myeloid differentiation factor 88 (MYD 88), Trif, and phosphorylated IL-1 receptor-associated kinase was significantly increased in diabetes type 1. There was increasing TNF- α and IL-1 β level in these patients. The expression of TLR2 and TLR4 was significantly associated with glycosylated hemoglobin.³

In this study demonstrated the expression of TLR2 mRNA was higher in group of treatment with tuberculosis and hyperglycemia which was examined in fifth week after treatment compared to a group with tuberculosis without hyperglycemia.

b. Coronin -1A level

Coronin-1A is regulated by Phox protein through deployment of 'C' protein kinase to phagosome then subsequently occur phosphorylation P47phox and Coronin complex. Coronin phosphorylated dissolved and disposal of Coronin layer cause fusion of phagosome with endosome. Cholesterol, epigallocatechin-3-Gallate (EGCG, the main component in green tea polyphenol), vitamin D3 + retinoic acid and chenodeoxycholic acid + retinoic acid regulate Coronin-1A gene transcription. Through receptor-Ck-dependent signaling, cholesterol is known to regulate SREBP and PPAR- γ transcriptional factor. EGCG was known to work through SP-1 transcription factors, in which vitamin D + retinoic acid and chenodeoxycholic acid + retinoic acid is known to work through VDR / RXR and FXR / RXR heterodimers. This stage showed epigenomic Coronin-1A gene control plays a role in the evolution of micobacterial phagosome.⁵

In this study, an increase of Coronin 1A level in group with tuberculosis and hyperglycemia were examined in the fifth week compared with the other treatment groups, showed the length of tuberculosis suffer and accompanied by hyperglycemia causes Coronin 1A level increased. This situation blocked fusion of phagosome and lysosome so phagocytosis against micobacterium not occur, which

increased the number of bacterial colonies and also increased tuberculosis severity.

Studies with type 1 diabetic mice induced by alloxan showed that neutrophils and alveolar macrophages impaired in cytokine secretion and phagocytosis, but with insulin administration can recover some of the circumstances. The main finding in chronic diabetes was on immune response to mycobacteria by slowing natural immune response that mediate production of interferon- γ . This effect is caused by disorders of rapid inflammatory response by infected alveolar macrophages, while dendritic cells migration from lungs to local lymph nodes.⁸

5. Conclusion

It could be concluded there were increasing of TLR2 mRNA expression and Coronin-1A level in lung mice which were infected with M.tb and hyperglycemia examined in the fifth week compared with other groups of treatment. This may be due to many inflammatory factors that act as ligands of TLR that can spur an increase in the expression of TLR especially TLR2.

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