Immunological Aspects of Latent Tuberculosis Infection in Diabetes Mellitus

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Abstract
The interaction of diabetes mellitus (DM) with tuberculosis (TB) is currently a health concern. Diabetes mellitus is one of the main risk factors for TB infection to become latent TB and / or progress to active TB. Immune mechanisms contribute to this increased risk. The disruption of the mycobacteria recognition process, phagocyte activity and cellular activity will affect the disruption of cytokine and chemokine production. Hyperglycemia that occurs will result in delayed adaptive immune response resulting in reduced Th1, Th2, and Th17 cells as well as the cytokines produced by these cells that play a role in macrophage activation and TB inflammatory response. Understanding of the immune mechanisms that underlie the sensitivity of DM to TB infection, especially latent TB, will facilitate the implementation of strategies in screening and therapy to deal with the double burden of both diseases. The purpose of this literature study focuses on the relationship of DM with latent TB infection in terms of immunology. (J Respirol Indones 2021; 41(4): 289–300)

Keywords: DM, tuberculosis, latent tuberculosis infection (LTBI), immune mechanism

Aspek Imunologis Tuberkulosis Laten pada Diabetes Melitus

Abstrak

Kata kunci: DM, tuberculosis, infeksi TB laten, mekanisme imun

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INTRODUCTION

Diabetes mellitus (DM) is one of the non-communicable diseases whose number of cases is increasing. Currently, it is estimated that there are more than 350 million cases of DM in the world; most of them are type 2 DM (DMT2). This number is predicted to continue to increase to 592 million cases in 2035. The number of people with DM in Indonesia has been estimated at more than 5 million, it means that 1 in 40 Indonesian people suffer from diabetes. Based on Riset Kesehatan Dasar (Risksdas) 2018 data, the prevalence of DM in Indonesia increased from 6.9% in 2013 to 8.5% in 2018. World Health Organization (WHO) data shows that 8 out of 10 countries with the highest incidence of DM are also countries with the highest TB sufferers. Indonesia as a country that ranks 2nd in the number of TB sufferers, is also included in the 10 countries with the highest number of people with DM in the world.1–3

Diabetes mellitus is known to increase the risk of infection, including TB infection. Several studies have also shown that DM is an important risk factor for TB infection. The cohort studies found that the relative risk of developing active TB in people with DM was 3.1 (95% CI 2.3–4.3), and some case-control studies had an odds ratio (OR) of 1.2–7.8. In general, WHO data shows that DM will increase the risk of TB infection by 3 times greater than the average population.4,5

Primary M.tuberculosis infection will develop into TB disease in 5–10% of individuals, and the remaining 90–95% will develop latent TB infection. In individuals with impaired immune response, both by exogenous and endogenous factors, M.tuberculosis bacteria remain in the host's body without causing clinical manifestations or what is known as latent TB. Latent TB infection (ITBL) is the largest reservoir for TB bacteria.4,5 Latent TB is a subclinical M.tuberculosis infection without clinical signs, bacteriological and radiological manifestations, but positive immunological test results (tuberculin or interferon-gamma release assay). Latent TB infection is important because individuals with latent infection can develop active TB at any time, even years after primary infection. Several studies have found that 2–15% of individuals with ITBL will develop active TB. It will certainly be a source of infection that will disrupt the global TB elimination program in 2050, which WHO has proclaimed. Currently, it is estimated that one-third of the world’s population, or 2–3 billion people has latent TB.4,5 Research conducted by Patra et al. on 72,684 individuals in 14 countries with high TB rates found that DM is one of the factors that lead to TB reactivation in addition to smoking, alcohol consumption, and low body mass index (BMI).6

Latent Tuberculosis

TB infection begins when the M.tuberculosis bacteria is inhaled, which will replicate during a certain period, followed by an immunological process. This process is latent TB, which is an infection characterized by persistent live bacteria in the host's body without evidence of clinical or radiological manifestations of active TB. Currently, the global number of latent TB cannot be calculated with certainty, but it is estimated third of the world's population has been infected with TB (2–3 billion people). It is estimated that 2–15% of these infected individuals have the potential of developing active TB.7,8

TB Pathogenesis

The course of TB infection is more complex than that of other pathogenic bacterial infections. TB infection has a more extended incubation period, and clinical outcomes are highly dependent on the host and pathogen. Tuberculosis can infect all organs, but the organ most affected is the lungs (60–75% of all cases) and is also a source of infection transmission, especially from cavities that form in the lungs. Active TB patients transmit M.tuberculosis bacteria through coughing, which will release droplet nuclei with a size of <5 μm. These droplet nuclei can survive for several hours in non-flowing air and then be inhaled by other individuals who contact with the patient. Approximately 30–50% of individuals in
household contact with TB patients will develop immune sensitization due to infection.\textsuperscript{7,8}

One droplet nuclei contain 1–10 \textit{M. tb} bacilli, which will then enter the lungs. Droplet nuclei can avoid the defense system in the bronchi because of their small size and can penetrate the terminal alveoli, which macrophages and dendritic cells will then phagocytose. \textit{Mycobacterium tuberculosis} can also infect non-phagocytic cells in the alveolar space, such as M cells, alveolar endothelial cells, and type 1 and 2 pneumocytes. In the early stages of infection, \textit{M. tb} is phagocytized by phagocytic cells and then replicates intracellularly and then immune cells that already contain \textit{M. tb} bacteria penetrates the alveolar cell barrier causing systemic spread. Intracellular replication and systemic spread continue to reach pulmonary lymph nodes and other extracellular sites until an adaptive immune response develops. This proves that \textit{M. tb} has an extraordinary ability to evade the body’s defense system and survive indefinitely in the host's body.\textsuperscript{7,8}

Infected individuals will generally develop an effective cell-mediated immune response within 2–8 weeks after infection that will stop \textit{M. tb} bacteria from replicating. Furthermore, activated T lymphocytes, macrophages, and several other immune cells will form granulomas which are walls of necrotic tissue that continue to expand and prevent the spread of bacilli. \textit{Mycobacterium tuberculosis} is generally eradicated in caseous granuloma tissue. Further development of the disease will be restrained. However, \textit{M. tb} bacteria will not be completely eradicated. In some individuals, these pathogens can develop effective strategies to evade the immune response to produce some bacteria that can survive and persist but do not replicate. It is known as ITBL, as evidenced by the presence of \textit{M. tb} in culture and the finding of \textit{M. tb} DNA in the lung tissue of patients who died not from TB disease, and pathologically, no signs of TB were found. Recent results show that TB infection can persist for up to 30 years or even a lifetime.\textsuperscript{7,8} The presence of defects in the immune system will result in the reactivation of dormant \textit{M. tb} bacteria after infection many years ago into active TB (TB disease).

**TB Immunopathogenesis**

Experts have agreed that the humoral immune system has no role in TB; on the contrary, it is the cellular immune system that plays a role in TB. \textit{Mycobacterium tuberculosis} is one of the intracellular bacteria that are facultative intracellular.\textsuperscript{8,9}

One of the characteristics of facultative intracellular bacteria is that they can live and even reproduce in phagocytes. These bacteria find a place to hide, so that circulating antibodies cannot reach it. Types of bacteria, such as \textit{M. tb}, inhibit phagocytosis and the formation of reactive oxygen intermediates (ROI) or the occurrence of respiratory bursts (oxidative). Bacteria can avoid phagosome traps so that they remain free in the cytoplasm and avoid further destruction.\textsuperscript{8,9}

**Specific Immunity In \textit{M. tb} Infection**

The studies conducted both in experimental animals and in humans have shown a wide-scale immune component in \textit{M. tb} infection. The cells that play a role in addition to macrophages and dendritic cells are CD4+ and CD8+ cells, CDI restricted T cells, γδ-T cells, and cytokines produced by these immune cells. Among the many immune cells that play a role in \textit{M. tb} infection, the most important are CD4+ and IFN-γ. CD4+, CD8+, and NK cells are the main cells that produce IFN-γ. The study of Carusso and Cooper cited by Dutta in mice with CD4+ deficiency showed that the production of IFN-γ by CD4+ early in infection accompanied by macrophage activation was a determining factor in the outcome infection.\textsuperscript{10} In addition, CD4+ cells also play a role in defense against \textit{M. tb} infection beyond their ability to produce IFN-γ. Decreased CD4+ count is strongly associated with reactivation of infection in chronic \textit{M. tb} infection.\textsuperscript{10} It was shown in the study of Scanga et al. cited by Schwander in \textit{M.tb}-infected mice, and reduced CD4+ counts resulted in worsening of pathological features and increased mortality of mice even though IFN-γ levels remained high as a result of normal CD8+ cell
responses and inducible nitric oxide synthase (iNOS) levels.\textsuperscript{11}

CD4+ T lymphocytes have an essential function in controlling infection in granulomas. Apoptosis is one of the important functions through Fas/Fas ligand interactions, production of cytokines (IL-2 and TNF-\textalpha{}), inducing other immune cells such as macrophages and DC cells to produce several immunoregulatory cytokines such as IL-10, IL-12, IL-15, and macrophage activation directly via CD40 ligand. CD4+ cells also play an important role for CD8+ cells in carrying out their function as cytotoxic cells mediated by IL-15. CD4+ cells can also control the growth of intracellular \textit{M.\textit{tb}} via nitric oxide mechanism.\textsuperscript{10,11}

CD8+ T lymphocytes also produce IFN-\gamma and several other cytokines that are also cytotoxic to \textit{M.\textit{tb}} present in infected macrophages. \textit{Mycobacterium tuberculosis} in macrophages will be killed by CD8+ cells directly with granulysin and ultimately facilitate the control of both acute and chronic infections. A large and excessive number of \textit{M.\textit{tb}}-specific CD8+ cells in ITBL individuals suggests that CD8+ cells play an important role in latent TB infection and supports the evidence for TB reactivation followed by CD8+ cell depletion, as shown by the Cornel model in latent TB.\textsuperscript{10-12}

Immune cells that play a key role in protection against \textit{M.\textit{tb}} infection are IFN-\gamma. Research has shown that humans and experimental animals with a defect in the IFN-\gamma gene receptor will be more susceptible to \textit{M.\textit{tb}} disease. Interferon-\gamma is produced mainly by CD4+, CD8+, and NK cells that work synergistically with TNF-\textalpha{} to activate macrophages to kill intracellular \textit{M.\textit{tb}} bacteria. Interferon-\gamma also increases antigen presentation and recruitment of CD4+ cells and CD8+ cells to kill \textit{M.\textit{tb}} bacteria and prevents memory T cells’ weakening. Interferon IFN-\gamma also induces more than 200 genes in macrophages, including genes expressing MHC class II and free radicals and nitric oxide production. The main mechanism of the antimicrobial effect of IFN synergizing with TNF-\textalpha{} is the induction of nitric oxide production and other reactive nitrogen intermediates (RNI) by macrophages through iNOS.

Some \textit{M.\textit{tb}} antigens such as lipoprotein 19kD can attenuate IFN responses to macrophages through inhibition of transcription of IFN-\gamma responsive genes.\textsuperscript{10-12}

Tumor necrotizing factor-\textalpha{} is a cytokine other than IFN-\gamma, which plays an important role in protecting against \textit{M.\textit{tb}} infection. Tumor necrotizing factor-\textalpha{} is produced by macrophages, DC cells, and T cells. TNF-\textalpha{} is produced by infected macrophages and then induces the expression of chemokines, including IL-8, MCP-1, and RANTES, which signal immune cells to migrate to the site of infection \textit{M.\textit{tb}}. The mice with reduced TNF-\textalpha{} or TNF-\textalpha{} receptors were more susceptible to \textit{M.\textit{tb}} infection. TNF-initiates migration, and granuloma formation is formed. The weaknesses TNF-\textalpha{} response will increase the number of \textit{M.\textit{tb}}. In resistant strains of \textit{M.\textit{tb}} (W Beijing family), phenolic glycolipids found in bacterial cell walls can inhibit the release of pro-inflammatory cytokines from macrophages such as IL-6, IL-12 including TNF-\textalpha{}. The IL-12 cytokine is also one of the important cytokines in the immune response to \textit{M.\textit{tb}}. Interleukin-12 activates CD4 cells to produce TNF-\textalpha{} and IFN-\gamma. The studies have shown that individuals with impaired IL-12 production have an increased risk of TB.\textsuperscript{11,12}

\textbf{\textit{M.\textit{tb}} Infection Persistency and Reactivation}

Three conditions may occur when we look at the natural course of \textit{M.\textit{tb}} infection, after initial infection, and sensitization. These conditions are influenced by predisposing factors, which will later determine infection and change the proportion in each part of the condition. After infection, there is a critical period in which predisposing factors determine the outcome. In the first group, the primary infection will progress too progressive. It only occurs in a small proportion of the adult population and is more common in the severely immunosuppressed group and infants. In the second group, the primary infection was completely controlled, and reactivation was unlikely. The third group of controls for unstable infection can be slow or even increase due to precipitating factors. It is in this group that reactivation is most likely to occur.
Precipitation factors can make the disease progression; before this occurs, a sub-clinical infection phase occurs first. In this third phase, *M. tb* can be isolated by culture and pathological changes seen from imaging, all of which precede clinical symptoms.\textsuperscript{10,12}

*M. tb* infection is characterized by the inability of the individual to develop a full immune response to eliminate the pathogen. *M. tb* bacillus has several strategies to avoid and manipulate host immune cells so that *M. tb* bacilli are avoided from host immune elimination. The result is that the pathogen can remain in the host cell. Several *M. tb* antigenic factors such as ManLAM and 19-kDa lipoprotein have been known to modulate the antigen presentation pathway and blunt the antimicrobial function of immune cells, including macrophages and other immune cells and RNI and inhibit phagolysosomal maturation. Several studies have been conducted to determine the persistence of *M. tb* bacteria in host cells, but these studies have only been carried out on experimental animals. Several factors have been identified as the cause of *M. tb* persistence, including phospholipases with codes plcA, plcB, plcC and plcD, PhoP and PhoQ proteins, and phosphatase binding proteins PstS1 and PstS2.\textsuperscript{10}

Active tuberculosis may develop immediately after exposure and after primary infection or during ITBL. ITBL reactivation is a state where *M. tb* is active again from its dormant period. Several factors that can trigger active disease from an inactive infection include HIV, which is the main factor in reactivation of latent TB, in addition to uncontrolled diabetes, malnutrition, old age, kidney failure, or diseases with the use of immune-suppressing drugs such as cancer and rheumatism. TB reactivation can be found in all body organs that were the site of primary infection PstS2.\textsuperscript{12}

Research conducted by Sun et al. cited by Dutta et al.\textsuperscript{10} showed that adding a supernatant containing acid-labile and heat-stable resuscitation factor increased the viability of the *M. tb* H37Rv culture. This finding continued with discovering the protein resuscitation-promoting factor (Rpfs), which is thought to be associated with the reactivation of *M. tb* from a previous chronic infection. The *M. tb* genome containing the Rpf gene encoding rpfA–rpfE was shown to stimulate the regrowth of nonreplicating *M. tb* cells in vitro and increase the survival of *M. tb* bacilli in vivo.

Traditionally ITBL is an *M. tb* infection that resides in foci in the granuloma in a non-replicating state and will cause active TB when the immune response is compromised. From the model, it is known that during infection, *M. tb* will grow well in phagosomes. Still, there are some bacteria from necrotic macrophages that escape into the extracellular environment and stop replicating. The cessation of bacterial growth occurs even though a complete immune response has not occurred due to creation of a hypoxic and acidic extracellular environment and the release of bactericidal enzymes from dead macrophages and neutrophils.\textsuperscript{10,12}

From the ITBL model, it is also known that foamy macrophages arise during the chronic infection process, which further phagocytizes cellular debris rich in fatty acids and cholesterol originating from cellular membranes. Foamy macrophages are full of non-replicating bacteria. Then the granuloma in the lung is pulled into the bronchial tubes and back to a different location in the lung parenchyma so that the infection process occurs again in the new site. In this dynamic process, reinfection in the upper lobes of the lung leads to the possibility of cavitation. The high oxygen tension in the location supports the rapid growth of extracellular *M. tb* bacilli, and the host immune response can fully control it. The subsequent stronger inflammatory response will lead to tissue destruction, liquefaction, and cavity formation. This dynamic infection process is similar to the development of immune reconstitution inflammatory syndrome (IRIS) in patients with HIV. HIV patients will tolerate the existing *M. tb* bacilli because the host immune response is unable to control the growth of the pathogen, but as the CD4+ cell count increases due to antiretroviral
administration, a granuloma response and active TB rapidly develop.\textsuperscript{10,12}

**Location of M.Tb Basis In Latent TB**

In latent TB, it is known that there is a small proportion of \textit{M.tb} bacteria that remain alive which will later be able to reactivate into active TB. However, the exact location or place of the \textit{M.tb} bacillus is still a question. Several studies have concluded that \textit{M.tb} bacteria in latent TB are present in caseous tissue and necrotic granulomas. Rabinowitsch's study cited by Dutta showed that lymph nodes in mesenteric and bronchial origin from patients without active TB containing lime-like tissue were found to cause TB when infected in rabbits; this occurred in 4 out of 5 experimental animals. This study, along with other similar studies, put forward the theory that the spread of \textit{M.tb} bacilli in the lymphatic system is important in the adaptive immune system and is also important as a basis for the pathological spread of infection.\textsuperscript{10,12}

Research Hobby et al. cited by Dutta, who performed culture in liquid medium of 85 closed healthy necrotic lesions in 40 treated TB patients found that 78% of these lesions could grow \textit{M.tb}, but the viability of the bacilli was only detected after an extended incubation period of 9-12 weeks.\textsuperscript{10}

**Latent TB Risk Factors**

Individuals with untreated active TB are a source of transmission of new TB infection cases. The source of transmission comes from the respiratory tract of patients with active TB. Controlling the transmission of TB infection from active TB cases is one of the main objectives of the TB eradication program in countries with high TB rates, including Indonesia.

Generally, infected individuals will experience an infectious process limited by the immune system, and \textit{M.tb} bacteria will survive in granuloma caseosa or tubercles. It is estimated that 5% of infected individuals will become ill with TB in the first 2 years after infection. Approximately 10% of infected individuals will develop latent TB, which is reactivation in the first 1 year after infection, and this risk persists for life. This reactivation generally occurs by reactivating TB bacteria that were previously dormant in the primary infection or because of a lesser chance of being infected with TB bacteria again. Overall, approximately 10-15% of these infected individuals will be at risk of developing TB disease at some stage of their life. This risk will increase to 10% per year in HIV-positive individuals and other immunocompromised individuals, including DM.\textsuperscript{10,12}

There are two different essential aspects of the risk of TB infection, namely the risk of infection and the risk of progression of infection to TB disease. The risk of infection when exposed to the causative bacteria is mainly regulated by exogenous factors with a combination of intrinsic factors such as the infectious level of the source of transmission, the proximity of contact with the source of transmission and social risk factors and habits such as smoking, alcohol and indoor air pollution.\textsuperscript{13}

Factors that increase the progression of infection to TB disease are influenced more by factors that exist in the host itself. Conditions that can alter the immune response, such as HIV, become very important and decisive on this factor. Still, on the population, the impact is highly dependent on the local prevalence in the area, but other factors such as diabetes, smoking habits, malnutrition and indoor air pollution are also factors that have a more significant impact on the population in accelerating the risk of progression of TB infection.\textsuperscript{6,13}

**Diabetes as a Risk Factor for Latent TB**

Several studies have shown that diabetes is one factor that increases the risk of developing active TB. In a systematic review conducted by Jeon and Muray, it was concluded that individuals with DM had a three times greater risk of developing TB than individuals without DM.\textsuperscript{11} Epidemiological studies in India and Mexico show DM is present in 22% of TB cases or one-third of all TB cases in these countries. This, of course will have a significant impact on the TB control program.\textsuperscript{14,15}
Diabetes mellitus also has an impact on the clinical and course of TB disease. TB patients with DM more often show positive smear results than those without DM, contributing to the spread of TB infection. In addition, TB-DM patients have a higher mortality risk than TB without DM.\textsuperscript{5,14} The increased risk of TB infection in people with DM is thought to be due to changes in the immune response. Diabetes mellitus is characterized by hyperglycemia caused by impaired insulin secretion, impaired insulin response or a combination of both. Uncontrolled glycemic levels can lead to compromised immunity, making it easier for patients to be infected with intracellular bacteria, especially \textit{M. tb}.

The etiology of DMT2 includes a complex mix of genetic and environmental factors that lead to insulin resistance and elevated blood glucose and free fatty acids (FFA) levels. Changes in glucose and lipid metabolism in adipocytes and hepatocytes will increase the pro-inflammatory state characterized by an increased population of activated macrophages. Pressure on pancreatic beta cells occurs as a result of metabolic and inflammatory changes that will eventually lead to insulin deficiency and hyperglycemia.\textsuperscript{5,16}

The hyperglycemia will result in an impaired immune response to \textit{M. tb}. Research conducted in Japan showed a relationship between glucose intolerance and the incidence of TB. This study is in line with a survey conducted in Africa with the results of the risk for TB in subjects with glucose at 11.1 mmol/L was 2.15 times compared to subjects with glucose at <11.1 mmol/L.\textsuperscript{10} Hyperglycemia is known to affect macrophage action. Macrophages themselves are one of the first immune cells to fight mycobacterial infection and are the cells in which mycobacteria thrive during infection.\textsuperscript{16}

Hyperglycemia also results in impaired macrophage function in receptor expression associated with antigen presentation and T cell activation. Research conducted by Lopez-Lopez et al. showed that macrophages of diabetic patients infected with H27Rv TB had reduced expression of CD86, CD80, HLA-DR, and molecules associated with antigen presentation and T lymphocyte activation as reduced induction of IL-6, IL-1\textbeta, IL-10 and IL-12 before and after infection.\textsuperscript{17} Glycemic control is usually measured by the HbA1C level, which indicates glucose concentration in the blood 2 or 3 months before the test.

HbA1C levels recommended by the American Diabetes Association (ADA) are <7% or preprandial capillary glucose levels 83-130 mg/dL and postprandial glucose levels <180 mg/dL. Kumar et al. showed changes in the levels of monocyte activator markers.\textsuperscript{18} Almeida et al. demonstrated that high HbA1C levels were associated with the severity of lung damage by \textit{M. tb}, but the mechanism that explains this pathology is still unclear.\textsuperscript{19} In in vitro studies, it is known that \textit{M. tb} uses triglycerides from the host as a site of infection in hypoxic conditions.\textsuperscript{20} This causes the formation of a fatty acid-rich environment and becomes a source of energy from lipids during \textit{M. tb} infection, as seen in experimental animal granulomas. This concept is consistent with latent TB infection in DM. Lipid accumulation in macrophages will form foam cells (foamy cells) that secrete cytokines. Foamy macrophages contribute to mycobacterial persistence and pathological changes in tissues during TB. High triglyceride levels in DM patients will cause increased levels of oxidized LDL (Ox LDL).\textsuperscript{21}

Research conducted by Vrieling et al., shows that macrophages with high levels of Ox-LDL have a high mycobacterial load compared to macrophages that Ox-LDL does not accompany.\textsuperscript{21} This evidence reinforces the concept that high Ox-LDL concentrations in DM patients contribute to the progression of TB infection in DM, and dyslipidemia in DM is strongly associated with DM susceptibility to TB infection. Kumar et al. showed that DM patients with latent TB and active TB had decreased levels of adiponectin, adipisin, and/or increased levels of leptin, visfatin and PAI-1.\textsuperscript{22} Adiponectin and adipisin were negatively correlated with HBA1C levels, while visfatin, leptin and PAI-1 were positively correlated with Hba1C levels. These changes in systemic adipocyte levels strongly indicate systemic inflammatory changes in adipose tissue in people.
with diabetes, thus contributing to the course of TB infection. Resistin is a protein that is considered to play a role in insulin resistance both in humans and in experimental animals. Thus, protein is key to the link between obesity and diabetes. Resistin increases the expression of proinflammatory cytokines such as TNF-α, IL-6, IL-12 and MCP-1, macrophages and hepatic stellate cells through the NF-κβ factor pathway. Research shows that people with diabetes have high resistin levels in serum along with a reduced ability of THP-1 macrophages to produce ROS when fighting *M.tbc* infection. TB infection showed a marked increase in resistin levels. This evidence suggests that TB induces changes in resistin production that affect metabolic and immune responses and is derived from deactivated macrophages.\(^\text{16,22}\)

Treatment given to DM patients aims to overcome hyperglycemia. The standard drug recommended by the ADA is metformin. Several retrospective studies conducted in Taiwan showed hyperglycemia therapy with metformin was a factor in preventing TB in DM patients (HR 0.552; 95% CI (0.493 to 0.617) and HR 0.84; 95% CI (0.74 to 0.96)).\(^\text{23}\) Metformin works by reducing MMP production 1, 2 and 8. Metformin is associated with a reduced number of mycobacteria in TB patients with DM; however, Lee et al. in Seoul showed that the use of metformin and anti-tuberculosis drug (ATD) in DM-TB patients had no effect on sputum conversion and observed TB recurrence for 1 year after completion of therapy.\(^\text{23}\) These results are supported by animal studies which concluded that there was no increase in the efficacy of ATD therapy with the addition of metformin. The evidence above shows that the control of hyperglycemia exerts an influence on the metabolic environment that can reduce susceptibility to TB infection through its mechanism.

Hyperglycemia conditions in the long term accelerate the formation of advanced glycation end products (RAGE) produced by non-enzymatic protein glycation. Increased levels of AGE and FFA will trigger the production of inflammatory mediators and reactive oxygen species (ROS). The increase in ROS is also due to increased glucose metabolism through oxidative phosphorylation. There is a balance of ROS production in healthy individuals with an increase in antioxidant activity, especially that carried out by glutathione, but this is not the case in people with diabetes. Obesity is also thought to influence chronic inflammation due to increased pro-inflammatory cytokines by adipocytes and macrophages in adipose tissue. Excessive production of TNF-α in adipose tissue is associated with inflammatory, metabolic changes, and it is also a cause of insulin resistance. Macrophages that have not been activated will accumulate in adipose tissue, releasing inflammatory mediators such as TNF-α, C-reactive protein (CRP), IL-1β, IL-6, IL-8 and IL-12. Many other mechanisms influence changes in the immune response of DM patients to TB infection that have not been studied extensively, including age, vitamin D levels, and the anti-inflammatory effect of the drugs used.

Natural and Adaptive Immunity Against TB in People with DM

In people with DM, the disruption of the immune process has started during the initial process of introducing *M.tbc* by the innate immune cells of the host. Monocytes of diabetes patients experienced a significant decrease in their attachment function and phagocytosis to *M.tbc* compared to monocytes of non-DM patients, due to changes in monocytes with DM, especially in the C3 component, which is a complement to *M.tbc* phagocytosis events.\(^\text{13,14}\) This is also the case, following the study of Martinez et al.,\(^\text{54(39) quoted from 24}\) which showed a decrease in the phagocytic function of macrophages in rats after being infected by *M.tbc* for 2 weeks. A multivariate analysis comparing monocytes of TB DM patients with non-DM TB found an increase in CCR2 and CCL2 (MCP-1) expression, which are CCR2 ligands in DM patients. In addition to the reduced ability to perform phagocytosis, people with diabetes also show reduced gene expression that contributes to the presentation of antigens and antimicrobial peptides.\(^\text{13,14,22}\)
The process of phagocytosis and the initial response to prepare for the adaptive immune function is an essential process in the host immune response to limit the growth of *M. tb*. The delay in the phagocytic process that occurs in people with DM facilitates *M. tb* infection and persistence. Research by Restrepo et al. showed that TB patients with DM showed an increase in the number of NK cells found in the blood and bronchoalveolar lavage (BAL) fluid compared to the non-DM TB group. However, the effect of this on the sensitivity of DM patients to TB infection is still unclear.

Dendritic cells are immune cells that play a role in the relationship between natural and adaptive immunity. Migration of dendritic cells to lymph nodes is essential in TB infection. Research has revealed that DM patients with TB infection show lower myeloid and plasmacytoid dendritic cells than normal individuals. However, their contribution to the pathogenesis of TB infection in DM is still unclear, although it is possible that hyperglycemia can affect it.

Neutrophils are immune cells that also play an essential role in the pathogenesis or defense against TB infection. Neutrophils play a role in innate immunity against TB through an oxidative process that kills mycobacteria. Neutrophils are the first immune cells to migrate to the site of infection and will secrete cytokines and chemokines, which in turn induce and activate other immune cells. Hyperglycemia that occurs in DM has been shown to increase integrins' adhesion and expression, reducing the chemotaxis and microbicidal activity of neutrophils. In addition, there is evidence that glycated collagen inhibits neutrophil migration due to the RAGE receptor expressed by neutrophils and other leukocytes.

Natural Killer (NK) cells are also effector cells of innate immunity. During the early stages of infection, NK cells can activate phagocytic cells to the site of infection and rapidly recognize and destroy infected host cells. In addition, NK cells produce several cytokines, including IFN-c, IL-17 and IL-22, which play an essential role in the host defense mechanism against mycobacterial infection. Recent studies have shown that TB DM is characterized by increased expression of type 1 (TNF-) and type 17 (IL-17A and IL-17F) cytokines from NK cells.

Antimicrobial peptides are critical components of innate immunity against pathogens and are primarily present in phagocytic cells. Several studies have shown that antimicrobial peptides with high antimycobacterial activity but low immunogenic properties are promising therapeutic agents. Among several peptides studied, it is known that cathelicidin LL-37 is one of the antimicrobial peptides that responds very well to *M. tb*. Gonzales-Curiel et al. showed a higher expression of cathelicidin in inactive TB compared to latent TB. Arliny concluded that the cathelicidin level obtained was higher in the active TB DM group than in the active TB without TB infection, the LL-37 cathelicidin level >30 ng/ml became one of the predictor factors in latent TB DM to active TB in addition to the HbA1c factor > 10 and a history of smoking.

Several studies conducted on TB patients with DM showed increased levels of Th1 (IFN-γ, IL-2), Th17 (IL-17A), IL-10, and decreased levels of T regulatory (CD4+, CD25+, CD127-). This suggests different regulation of immune responses in TB patients with DM and TB without DM. Several studies that tried to compare the immune response in the two groups turned out to get different results, so it is still unclear how this change in immune response occurs in the two groups. In latent TB, it is suspected that there are also changes in the natural and adaptive immune responses. However, very few studies explain this relationship, thus is difficult to obtain clear information.13,14,21

Cytokines types 1 and 17 and IL 1 are cytokines known to affect a person's susceptibility to TB. Nathella et al. stated that diabetes could alter the balance of these cytokine levels in latent TB infection. In DM, latent TB inflammatory cytokines type 1 (IFN, TNF and IL 2), 17 and IL 1 and other pro-inflammatory cytokines were reduced compared to those without DM. Other systemic pro-inflammatory cytokines such as IL-1β, IL-18 and IL-10 also decreased, but not type 2 cytokines. The
poor glucose control will affect the progression of latent TB to active TB. CD8+ lymphocyte cells are known to have a minor role in \( M.tb \) infection, but it is known that in latent TB DM subjects, the expression of cytokines involving CD8+ lymphocyte cells is also reduced.\(^{25}\) It can be concluded that DM will change the immune response by introducing CD4+ and CD8+ lymphocytes cells to be suboptimal and increasing the risk of developing active TB. In people with DM, progression to active TB results from impaired function of alveolar macrophages regardless of previous exposure to infection. Although this mechanism has not been thoroughly studied, there is evidence that 10–20% of latent TB have transcriptional signs of active TB from the peripheral blood; this supports the opinion that the latent TB subgroup may have clinically hidden but biologically active foci of infection and therefore have a high risk of developing infection progress to active TB.

Until now, there is no firm recommendation whether patients with DMT2 are given or not given latent TB prophylaxis; unlike in other populations, ITBL prophylactic therapy has not been fully evaluated, no specific studies are determining the efficacy of preventive therapy against the onset of active TB in DMT2 patients, as well as a more in-depth study of the antimicrobial effect of drugs used to treat diabetes (metformin) or other antidiabetic drugs.

The susceptibility of DM patients to TB infection originating from immunity is not yet fully understood. The increased risk of TB infection in DM patients is caused by several factors, including the direct effect of hyperglycemia and insulin resistance and indirectly through the function of macrophages and lymphocytes. Impaired immune response in DM patients facilitates primary TB infection or reactivation of latent TB.

**Demographic Aspects of Latent TB in DM**

Data on the prevalence of latent TB in DM in Indonesia is unknown because there is only a few research data that measures this. Arliny obtained data that there were 33.9% latent TB in DM patients without complications of decreased kidney function.\(^{24}\) Koesomadinata obtained data on the prevalence of latent TB in DM patients of 38.9%. \(^{24}\) Data on latent TB in DM patients from other countries varied, 50% in Mexico, 28.2% in Singapore, and 28.5% in Malaysia.\(^{24}\) Almost all research obtained data that more male were diagnosed with latent TB than female, with the most age range being 53.4–59.5 years. From the research, it is known that the duration of DM is one of the risks of latent TB. Research conducted by Merza et al. found a significant relationship between the duration of diabetes 10 years with latent TB (OR 2.692; 95% CI 1.016–7.267).\(^ {95(43)}\) quoted from 24

Epidemiological studies conducted in the United States showed that patients with poor glycemic control had a 2.2 times risk of developing latent TB than patients without a history of diabetes. DM patients who have a fasting blood glucose >130 mg/dL have a 2.6 times risk of developing latent TB than patients without a history of diabetes.\(^ {122(44)}\) quoted from 24 Martinez et al., in their study, obtained a mean HbA1c in latent TB diabetic subjects of 7.5%. This value is lower than that obtained in Arliny's research, which is 8.4%,\(^ {24}\) quoted from 24

Arliny obtained data that latent TB DM had a lower BMI than DM without TB infection and higher than active TB DM.\(^ {24}\) There is a significant difference in BMI between latent TB DM and active TB. One of the causes of the increased risk of latent TB in people with diabetes is the relationship between adipose tissue and \( M.tb \) replication. Adipose tissue constitutes 15–25% of the total body mass and is an active production site for hormones and mediators of inflammation. The increasing prevalence of obesity has led to a greater incidence of T2DM, and weight gain is usually (though not always) associated with T2DM. People with T2DM have a 2–3 times risk for infection and TB disease, so there may be a potential relationship between adipose tissue and disease pathogenesis. In addition to regulating energy homeostasis, adipose tissue is rich in macrophages and is involved in producing cytokines. Adipose tissue contains adipocytes, monocytes, and macrophages. It is
suspected that body fat tissue can provide a shelter for *M.tb* to hide and escape from the immune system. Research Ugarte-Gil et al. found that *M.tb* can reach into the fat cells and survive there. A large number of infections will cause *M.tb* to have large numbers in adipose tissue.

**CONCLUSION**

Diabetes can induce changes in the immune response to TB infection, both natural and adaptive immune responses. Disruption of the innate immune response followed by a hyperreactive adaptive immune response causes an increased risk of TB infection in diabetes. Although several pathophysiological and immunological pathways are known to play a role in diabetes, and ITBL as well as active TB, more in-depth research is still needed on screening and prophylactic therapy for latent TB in DM.

**REFERENCES**


