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The Effect of Vitamin D on the Lung Tissue Damage in Mice Infected with *Mycobacterium tuberculosis.*

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ABSTRACT

Eradication effort of tuberculosis disease in Indonesia has been carried out by implementing the strategy of Directly Observed Treatment Short-Course (DOTS) free of charge. However, its prevention and eradication are still unsatisfactory and Vitamin D expected to be able to enhance intracellular immune response. An experimental research using randomized post-test control group design was selected in this study. Twenty four mice were selected and allocated randomly into two groups. Twelve mice in one group were given 100ng of vitamin D orally, while twelve mice in another group were not given vitamin D. Then each group was allocated randomly into two sub groups of six mice. Six mice in one sub group were infected with *Mycobacterium tuberculosis*, while six mice in another sub group were not infected with *Mycobacterium tuberculosis.* The results showed that Vitamin D plays a role in increasing the body's resistance to tuberculosis and inhibits lung tissue damage due to tuberculosis

Keywords: Vitamin D, tuberculosis, lung tissue damage.

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INTRODUCTION

The incidence of tuberculosis in Indonesia was ranked fifth in the world (WHO, 2010), efforts to combat tuberculosis in Indonesia has been done using a strategy Directly Observed Treatment Short - Course (DOTS) with free treatment, but prevention and eradication is still not satisfactory.

The degree of damage to lung tissue can be used to assess the effectiveness of the drugs tested in experimental animals (Bast, 2004; Koendhori, 2008), but it also can be used to assess the virulence (Dormans, 2004). Lung tissue damage in Mycobacterium tuberculosis infection ranging form neutrophil aggregation, followed by proliferation and exudation of the pulmonary parenchyma (Robin SL, 1974), nevertheless granuloma is a sign of a chronic stage of infection with Mycobacterium tuberculosis as the host immune system efforts to localize further multiplication and dissemination to other organs (Ordway, 2005). Initial trip Mycobacterium tuberculosis infection varies greatly, depending on the immune factors, the sensitivity of the host and virulence or aggressiveness of the bacteria (Dormans, 2004). Tuberculosis lesions in the early course of the disease is proliferation and exudative. In individuals who are resistant to tuberculosis, phagocytosis reaction occurs in the formation of the boundary wall of fibroblastic and scarring. In individuals who are susceptible to tuberculosis exudative lesions will occur will be more extensive, involving many inflammatory cells and characterized the ability to locate the bad (Robins, 1974). Furthermore, in response to growing bacteria to grow acquired immune system (adaptive/acquired) with granuloma formation. Furthermore, response to bacteria is growing until acquired immune system (adaptive/acquired) with granuloma formation is gained.

Granulomas in mice differ with granulomas in human beings. Granuloma in mice composed by neutrophils, macrophages and lymphocytes by activated macrophages and lymphocytes structure that surrounds a collection of infected macrophages. In this granuloma no necrosis and Langhans type cells that are different histopathology (Cardona, 2000). Although the structure is different but the same function is to control the infection and prevent the spread of infection (Flynn JL, 2005). Not the appearance of granuloma necrosis in mice showed that the immune response in mice stimulated more strongly than in humans resulting in the spread of infection is lower (Cardona, 2000)

Vitamin D is imunoregulator beneficial for bone health that helps the absorption of calcium in order to keep bones strong, also proven to help maintain the immune system (Sebastian, 2008). The purpose of this study is to explain the effect of vitamin D on the lung tissue damage in mice infected with Mycobacterium tuberculosis.

MATERIALS AND METHODS

This study was an experimental study with post-test control group design. The study sample was male mice Balb / c, aged 8-10 weeks with a weight of about 20 grams. Mice were given food and drink ad libitum before and after infected with Mycobacterium tuberculosis (Mtb). Sample size of 24 mice was divided into 6 mice / group. This study was approved by the ethical feasibility of the Research Ethics Committee of the Faculty of Veterinary Medicine, Airlangga University, Surabaya, Indonesia.

Making the colony suspension of Mycobacterium tuberculosis

An amount of 1.5 ml of sputum containing acid-resistent bacilli, to be entered into a sterile tube and mixed with 1.5 ml of 4-8 % NaOH. This mixture was stirred 1 minute and taken with a sterile loop and then planted on Lowenstein - Jensen. The tube was closed with cotton and paper and then coated with paraffin, then placed in an incubator at 37 °C for 4-8 weeks (Koneman, 2007). Mycobacterium tuberculosis colonies grown on Lowenstein - Jensen medium suspension was made by adding 0.9 % NaCl to obtain the raw concentration of 1 Mc . Farland, further diluted with 0.9 % NaCl to obtain a suspension of 0.2 mg / ml.

Animal models infected with Mycobacterium tuberculosis

Mice Balb /c, weight of 20-30 g, aged 8-10 weeks of acclimatization conditions with controlled room temperature 22-25ºC for 7 days. Do anesthesia in mice with phenobarbital 0.6 mg / kg intraperitoneally, then made a small incision in the midline so that it looks the trachea. 100 mL of the suspension was added to the trachea of mice with tuberculin needle in a vertical position (Dormans, 2004). Mice were maintained with

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ventilation and temperature controlled to get the manifestation of tuberculosis, mice were sacrificed at week 14 to analyze the histopathological tissue.

Provision of Vitamin D

Vitamin D is weighed about 0.1 mg , dissolved in 0.9 % NaCl ad 200 ml and 0.2 ml solution given (100 ng) orally (Lamire . JM , 1991) .

Preparation histopathology of lung tissue of mice

Lungs of mice were fixed with 10 % formaldehyde solution in PBS and then soaked in paraffin. Lung tissue was cut as thick as 5 µm for histopathological examination, then stained with hematoxylin eosin and Ziehl Neelsen for further examined histopathological damage lung tissue damage mice by a score of Dormans. The parameters used are the degeneration, necrosis, congestion, edema, inflammation, fibrosis, epithelial hyperplasia and granulation,

RESULTS AND DISCUSSIONS

Table 1: Results of examination of lung tissue damage of mice by Dorman's scores

Code	Group			
	PT	NT	PC	Placebo
1	0,83	0,47	1,45	0,25
2	0,43	0,51	1,45	0,43
3	0,74	0,54	1,02	0,23
4	0,14	0,68	1,02	0,14
5	0,19	0,20	1,08	0,08
6	0,91	0,54	1,14	0,77
average	0,5400	0,4900	1,1850	0,3167
SD	0,3333	0,1581	0,1918	0,2520

Description:

Positive Treatment (PT) = group that was given Vitamin D and infected Mtb
Negative Treatment (NT) = group that was given Vitamin D and not infected Mtb
Positive Control (PC)= group that was not given Vitamin D and infected Mtb
Placebo = group that was not given Vitamin D and not infected Mtb

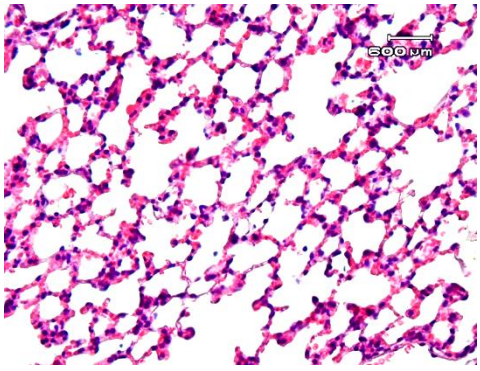

Fig 1 : Overview of normal lung from the group given Vitamin D and infected Mtb (HE staining : 400x magnification ; Olympus BX - 50 . Pentax optio 230 Digital Camera 2.0 megapixels) .

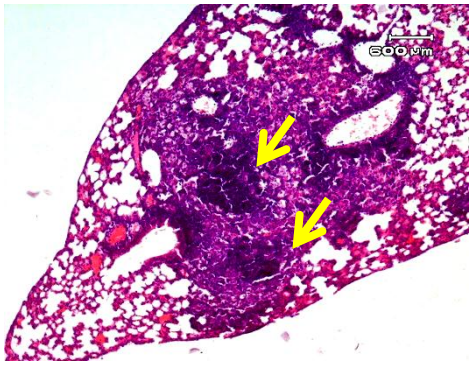

Fig 2 : Looks infiltration of inflammatory cells as well as the mass granulomas (tubercles) that indicates the presence of chronic infection (pneumonia Granulomatous) in the group that did not receive vitamin D and infected Mtb (HE staining : 400x magnification ; Olympus BX - 50. Pentax optio 230 Camera digital 2.0 megapixels) .

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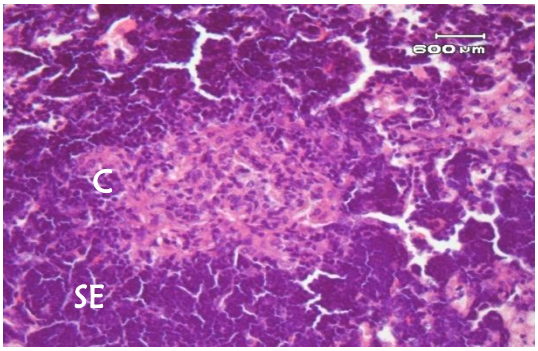

Fig 3 : characteristic of tuberculous granulomas characterized by areas of necrosis in the midst of granulomas (central necrosis / CN) , which is surrounded by epithelioid cells (SE) in the group that did not receive vitamin D and infected Mtb (HE staining : 400x magnification ; Olympus BX - 50 . Pentax optio 230 Digital Camera 2.0 megapixels) .

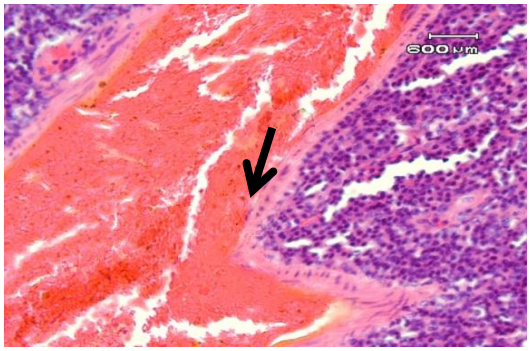

Fig 4 : Histopathological changes in the lung form of inflammatory cell infiltration and severe congestion (arrows) in the group that did not receive vitamin D and infected Mtb (HE staining : 400x magnification ; Olympus BX - 50 . Pentax optio 230 Digital Camera 2.0 megapixels) .

Data were analyzed Levene's test and Kolmogorov - Smirnov's test, and showed that the lung tissue damage in 4 groups of otherwise homogeneous and normal so that further tests are carried out analysis of variants (ANOVA test) . Results of ANOVA test showed the value of F = 5.383 and significance of 0.007. Test statistics continued to analyze different between groups, then carried Least Significant Differences test (LSD) , with the following results,

Table 2: Results of LSD damage lung tissue of mice between groups

Group (I)	Group(J)	Sig.
PT	NT	0.670
PT	PC	0.005
PT	Placebo	0.870
NT	PC	0.009
NT	Placebo	0.526
PC	Placebo	0.002

Test results of LSD were significant between the group given Vitamin D and infected Mtb (PT) and the group that did not receive vitamin D and infected Mtb (PC). This suggests there is formed granulomas in mice that did not receive vitamin D and infected with Mycobacterium tuberculosis. Characteristic of tuberculous granulomas characterized by necrosis of the middle period of the granuloma area (central necrosis / CN) surrounded by epithelioid cells (SE), multiple Langhans -type giant cells and lymphocytes evident in the control group were infected with Mycobacterium tuberculosis while in group given vitamin D and there is infected with Mycobacterium tuberculosis granuloma (Robbins , 1974) .

Granuloma consists of a collection of macrophages and epithelial lymphocytes are usually surrounded by a circle. Lymphocytes are always there either large or small quantities. Form of granuloma there are 3 groups: primary granuloma is granuloma characterized by the accumulation of macrophages in the central surrounded by lymphocytes. Granuloma secondary is located near the primary granuloma is an extension of the primary granuloma. Secondary granuloma lymphocytes characterized by a thick sheath surrounding the central part of granulomas and beyond many foamy macrophages. Tertiary granuloma is further development of secondary granuloma granuloma lesions which are combined with the most severe gradation (Cardona, 2000).

Macrophages play an important role in the immune response to Mycobacterium tuberculosis infection , so that when there is an infection with Mycobacterium tuberculosis , macrophages will mempersentasikan antigen , either MHC class I or class II to $CD4^+$ Th1 cells . Furthermore, $CD4^+$ Th1 cells would secrete IFN - γ to activate macrophages so as to kill the tuberculosis bacillus . γ interferon (IFN - γ) is a cytokine secreted by activated $CD4^+$ Th1 cells have immunomodulatory effects to multiple cells including macrophages (Wang, 2007) .

Granuloma is the main response of the chronic stage of infection with Mycobacterium tuberculosis which reflects the hard work of the immune response system to localize further multiplication and dissemination of the bacilli into cells and other organs. There is evidence that granuloma with a small diameter and an increase in macrophages showed that the immune response capable of controlling the growth of Mycobacterium tuberculosis . In contrast to the granuloma with a large diameter and a decrease in macrophages showed bacilli control ineffective (Ordway, 2005). Foamy macrophage function fagocyt central necrotic debris of the granuloma is a cell destruction occurs. After further work as a cleaner granuloma Foamy macrophages would leave room for the alveoli and encouraged coughed up or swallowed. Foamy macrophages are elements of inflammatory cells that are very important.

Granuloma growth depends on the proliferation of these cells because these cells are responsible for the incorporation of the lesion to form larger granulomas. The proportion of Foamy macrophages containing acid-resistant Bacilli number much so that it will lead to the formation of secondary granulomas (Cardona , 2000). Stimulation of macrophages or monocytes through heterodimer TLR - 2 / TLR - 1 lead to regulation of 1 - α - hydroxylase, but also in the vitamin D3 receptor (VDR) , then VDR generated from 25 - hydroxyvitamin in serum disinyalkan through certain VDR to encourage antimicrobial peptide chatelicidin and killing of intracellular Mycobacterium tuberculosis (Davies , 2006) . TLR signaling is essential for the manufacture of a complex containing protein kinase signaling leading to activation Nfk - β cause activation of transcription factors , so there Nfk - β active translocation to the cell nucleus (Billack , 2006) . In essence Nfk - ß stimulates transcription processes that produce interferon gamma (IFN - g) and interleukin 12 (IL - 12) (Gillmore , 2006) .

Tissue damage characterized by granulomas based on the DTH reaction (delayed type hypersensitivity) reaction caused tubercullin the person's reaction to tubercule bacilli. DTH process begins with Th - 1 cells and administration of antigen synthesis by macrophages. The reaction appeared at the time of 8-12 hours after infection. Redness occurs due to vasodilation with perivaskuler of CD4 T lymphocytes (Juanita, 2006). T cells will produce cytokines such as IL - 12, which then will activate other T cells and IFN - γ very important in activating macrophages (Davies, 2006). Granuloma has an important role in regulating the interaction between immune cells that lead to an effective response blocking and kill Mycobacterium tuberculosis , resist infection , prevent the spread of the organism, inflammation and tissue damage (Gordillo , 2005) .

Granulomas are formed because there intracellular Mycobacterium tuberculosis in macrophages infected or but not able to be killed by the macrophages , thus CMI will localize tubercle bacilli with bounded by T lymphocytes (Cardona , 2000) , but with increased levels of IFN - γ macrophages will be able to kill bacillus Mycobacterium tuberculosis , so there is no further processing of tissue damage . In contrast to the control group were infected with Mycobacterium tuberculosis granulomas because macrophages are not able to kill the tuberculosis bacillus or happening infected macrophages . Damage lung tissue is the increasing role of cellular immune responses with biomarkers of IL - 12 , IFN - γ and Nfk - β with the impact of lung tissue repair (Dormans , 2004). The results of this study provide information that giving vitamin D to give the effect of barriers to the formation of granulomas and prevent further pathological processes of the granuloma .

CONCLUSIONS

Vitamin D plays a role in increasing the body's resistance to tuberculosis and inhibits lung tissue damage due to tuberculosis and further clinical trials are still needed for the use of vitamin D as adjuvans in support for tackling cases of tuberculosis.

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