



THE RELATIONSHIP BETWEEN HIV HUMAN IMMUNODEFICIENCY VIRUS AND THE INCIDENCE OF TUBERCULOSIS (TB)

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ABSTRACT

HIV and Tuberculosis are a deadly combination, these two diseases accelerate the development of each other. The combination of these two infectious diseases influences each other in all aspects of the disease. Objective to determine the relationship between HIV (Human immunodeficiency virus) and the incidence of tuberculosis. Method: to determine the relationship between HIV (Human immunodeficiency virus) and the incidence of tuberculosis. Research methods : The research design used was analytical observational with a cross sectional approach. The population in this study were all HIV sufferers who were undergoing inpatient treatment in the UPIPI Room at Dr. RSUD. Soetomo. The sampling technique uses total sampling. The independent variable in this study is HIV sufferers. The dependent variable in this study is the incidence of tuberculosis. Data analysis used the Spearman Rank test with significance <0.05 . Result : The research design used was observational analytics with a cross sectional approach. The population in this study were all HIV sufferers who were undergoing inpatient treatment in the UPIPI Room at Dr. RSUD. Soetomo. The sampling technique uses total sampling. The independent variable in this research is HIV sufferers. The dependent variable in this research is the incidence of tuberculosis. Data analysis using the Rank Spearman test with significance <0.05 . Conclusions : Tuberculosis infection in HIV patients is closely related to a decrease in the immune system. As the stage of HIV increases, the body's immune system will decrease, so this can increase the risk of opportunistic infection with tuberculosis. The best way to prevent opportunistic infections is to avoid exposing HIV-infected people to tuberculosis.

Keywords: HIV/AIDS; opportunistic infections; tuberculosis

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INTRODUCTION

HIV infection increases susceptibility to tuberculosis (TB) by increasing the rate of progression from latent TB infection to active disease (Winter et al., 2020). Tuberculosis and HIV are a deadly combination, these two diseases accelerate the development of each other. The combination of these two infectious diseases influences each other in all aspects of the disease, starting from pathogenesis, epidemiology, clinical manifestations, treatment and prevention, and can even influence larger issues such as social, economic and political consequences. HIV-positive people are estimated to be 21-34 times more susceptible to active TB compared to HIV-negative people. Tuberculosis is also the main cause of death in HIV-positive patients. In 2016, 40% of HIV deaths were caused by TB. In 2016 in Indonesia, 360,565 cases of TB were found, with 14% of them known to be HIV-positive (WHO, 2017). Tuberculosis and HIV have the ability to reduce the work of the immune system, although until now this mechanism is not fully understood.

Human immunodeficiency virus (HIV) is a ribonucleic acid (RNA) virus that can cause a decrease in the host's immunity. In the world there are around 36.7 million cases of HIV, of which 1.8 million cases were only discovered at the end of 2016 (Pustil, 2017). HIV itself caused 1 million deaths worldwide in 2016. The highest HIV population is found in men who have sexual relations, sexual relations with men, people in prison, people who inject drugs, commercial sex workers, and transgender people. Indonesia is in fourth position in Asia as the country with the most HIV sufferers. Meanwhile, the incidence of HIV in Indonesia from 2014-2016 was recorded at 41,250 cases (Kemenkes, 2017). Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (Rottenberg et al., 2012). One third of people in the world are estimated to be infected with *M. tuberculosis*, but only 10-20% of them will develop active TB, while the rest remain asymptomatic or asymptomatic. Tuberculosis is one of the 10 causes of death worldwide. In 2016, 10.4 million had TB and 1.7 million died from the disease. India occupies the first position as the country with the most deaths due to TB in the world, followed by Indonesia in second position, China, the Philippines, Pakistan, Nigeria and South Africa. The number of TB cases discovered in Indonesia in 2016 was recorded at 298,128 cases (Kemenkes, 2018).

The mechanism of TB reactivation by HIV is by manipulating the macrophage bactericidal pathway receptor which is responsible for treating TB, degranulating chemotaxis, disrupting tumor necrosis factor (TNF) which mediates the apoptotic response in cells infected by *M. tuberculosis* (Tornheim & Dooley, 2017). Many other factors are also disturbed by HIV, such as interleukin-27 which will then reduce the activity of interferon- γ and interleukin-2 which will disrupt the control of these microbacteria in cells (Patel et al., 2007). HIV also interferes with the anti-inflammatory receptor deflazacort-6 which indirectly inhibits the clearance of mycobacteria by the immune system (Diedrich & Flynn, 2011). Other research shows that *M. tuberculosis* regulates the process of HIV infection and replication. In vitro research has proven that these microbacteria can increase HIV transmission in infected monocyte-derived macrophages (MDMs) to T cells and help replication by increasing the sensitivity of C-X-C chemokine receptor type 4 (CXCR4) (Kedzierska et al., 2003). Furthermore, monocytes from HIV sufferers show an impaired response to toll-like receptors (TLR) which also disrupts the maturation of MDMs including their phagocytic function. 8 When TNF is produced in response to infection by *M. tuberculosis*, it can also activate HIV replication in macrophages. Research also shows that the *M. tuberculosis* cell wall component, lipoarabinomannan (LAM) can activate HIV replication by inducing TNF and IL-6 resulting in activation of cell transcription. Human immunodeficiency virus on gp120 can attach to DC-SIGN while spreading throughout the organs. This results in HIV not being recognized as an antigen and inhibiting proinflammatory cytokines such as IL-2 and IL-10 (Tornheim & Dooley, 2017).

M. tuberculosis has been proven to provide a good environment for HIV growth, namely by increasing the activity of the CXCR4 coreceptor and C-C chemokine receptor type 5 (CCR5), increasing pro-inflammatory cytokines and decreasing the activity of C-C chemokine ligand type 5 (CCL5) (Liebert et al., 2006). That is why WHO recommends that every sufferer who is suspected or proven to be suffering from HIV should be examined to ensure whether or not there is TB infection in the sufferer. If after the examination is carried out and it is proven that the HIV sufferer is suffering from HIV, it is recommended to immediately carry out anti-tuberculosis drug therapy (OAT) (WHO, 2017). *Mycobacterium tuberculosis* and HIV potentiate each other, accelerating the decline in immunological function. Various strategies are currently needed to treat HIV-associated tuberculosis. Tuberculosis and HIV are two infectious diseases that receive special attention from health practitioners because their prevalence continues to increase. This also occurs in the incidence of TB-HIV co-infection.

This is because these two diseases and their causes can interact with each other and influence their respective epidemiology (Liebert et al., 2011). So, this research aims to determine the relationship between HIV Human Immunodeficiency Virus and the incidence of tuberculosis.

METHOD

The type of research that will be used in this research is observational analytic with a cross sectional study approach to determine the relationship between the incidence of HIV and TB. The research was conducted at one of the health services in Surabaya which handles HIV cases comprehensively with a number of holistic prevention and control programs. The sample for this study was HIV/AIDS patients who were undergoing inpatient treatment at the research site. The case sample is a portion of HIV positive patients who were hospitalized in March-April. The sampling technique in this study was carried out using a simple random sampling method. The research data sources used in this research are primary data sources and secondary data sources. Primary data sources were obtained from interviews, including variables of age, gender, occupation, drug risk factors, sexual risk factors, and the incidence of tuberculosis as proven by accurate examination results. Secondary data sources were obtained from patient medical records, including the variables age, gender. Data analysis techniques used univariate and bivariate analysis. Univariate analysis was carried out for each variable with the aim of describing the characteristics of each research variable. Bivariate analysis was carried out using the Rank Spearman analysis technique, because the data was on a nominal scale, the Confidence Interval (CI) was calculated with a confidence level of 95%.

RESULTS

Table 1.
Respondent characteristics (n= 40)

Respondent characteristics	Min	Max	Mean
Age	26	72	39.20
	f		%
Length of Suffering (Years)			
1	24		60
3	4		10
4	1		2.5
5	2		5
8	2		5
10	5		12.5
	2		5
Gander			
Male	30		75
Female	10		25
Work			
Private	31		77.5
Housewife	9		22.5
Risk factor			
Sex	24		85
Drugs	3		7.5
Sex and Drugs	3		7.5

Table 1, characteristics of respondents based on frequency distribution analysis, out of 40 respondents, it shows that the minimum age of respondents is 26 years and the maximum age is 72 years. Meanwhile, the characteristics of the duration of the sufferer show that 24 people (60%) have suffered for 0 years, gander shows that 30 people (75%) are male, and 10 people (25%) are female, occupation shows that 31 people (77.5%) worked in the private sector, and 9 people (22.5%) were housewives, risk factors based on respondent characteristics showed

that 24 people (85%) had had sex, 3 people (7.5%) had consumed drugs, while 3 people (7.5%)) engaging in sex and drugs.

Table 2.
Analysis of the Relationship between HIV and the Incidence of Tuberculosis (n= 40)

HIV	TB incidence				Total	
	Yes	%	No	%	f	%
Stadium 2	1	33.3	2	66.7	3	100
Stadium 3	10	62.5	5	33.3	15	100
Stadium 4	17	77.3	5	22.7	22	100
Total	28	70.0	12	30.0	40	100

Spearman's Rank Statistical Test p value 0.003

Table 2, the results of the analysis of the relationship between HIV and the incidence of Tuberculosis using the Spearman Rank test, there were 17 Stage 4 HIV sufferers who experienced TB incidents with a significance value of p value 0.003, which means there is a significant relationship between HIV sufferers and the incidence of Tuberculosis.

DISCUSSION

Based on the results of the characteristics of respondents based on age for TB co-infection with HIV, the average age of respondents was 39.20 years. In this case, this age is included in the productive age category (15-55 years). A study shows that the younger the person with HIV, the greater the risk of contracting opportunistic tuberculosis infection, this is because young HIV patients (≤ 30 years) tend to be more exposed to TB risk factors. Individuals with HIV have a weakened immune system, making them more susceptible to opportunistic infections such as tuberculosis (TB). The risk of developing TB in individuals with HIV is higher compared to the general population because the HIV virus reduces the body's ability to fight infection. At a younger age, the immune system is still developing and may not yet fully mature, so the risk of contracting opportunistic infections such as TB is higher. Additionally, younger individuals may also lack experience or understanding of the importance of HIV management and prevention, which may worsen their condition (Roselinda & Setiawaty, 2016).

Several other factors that cause opportunistic TB infections in HIV patients can be seen from several other factors such as the length of time they have suffered from HIV. Based on the research results, it was found that the majority of respondents had been suffering from HIV for 0 years or could be interpreted as several months, which means that there is a high susceptibility to TB because Their immune system is not yet fully strong because people who have just been diagnosed with HIV, especially in the first month, have not received the full benefit of antiretroviral therapy, which can cause TB germs to attack and spread in the body of HIV patients, thereby causing active or latent infections. Thus, we can conclude that long suffering from HIV is one of the causes of the increase in the incidence of TB and will worsen the condition of HIV/AIDs patients who require special attention in diagnosis, treatment and prevention (Framasari et al., 2020).

Characteristics of respondents based on gender in this study showed that the majority of respondents were male, 30 people (75%) and 10 people (25%) were female. This research is in line with that conducted by Blobel (2008) with the title "Survey of Characteristics of HIV-AIDS Sufferers in Makassar City in 2007" which stated that the highest number of HIV-AIDS sufferers was found in male sufferers. This is also in line with research Abdallah & Ali (2012)

who said that in Indonesia men are twice as likely to experience TB-HIV co-infection as women. Men tend to do activities outside the home more often than women. Women have fewer smoking habits than men. Smoking can cause the function of the cilia in the respiratory tract to be disrupted, which can increase the risk of being infected with tuberculosis. This can also improve the attachment of bacteria to respiratory epithelial cells, the result of which is bacterial colonization and infection. In summary, smoking may increase the risk of infection through ambiguous effects on the structure and function of the respiratory tract and the host's immunological response to infection (Bates et al., 2007).

Most people with HIV co-infection with Tuberculosis work in the private sector, amounting to 31 people (77.5%). Occupation reflects a person's socio-economic level, where if there are many people or HIV sufferers, the TB coefficient if they have a low economy will be closely related to unhealthy behavior such as smoking and inadequate health facilities. This is one of the things that can trigger tuberculosis co-infection in HIV/AIDS patients. People with low incomes tend to live in unhealthy environments such as slum housing, consuming less nutrition which can worsen the body's immune condition which results in susceptibility to tuberculosis infection (Marfidhotul Ifitah et al., 2020). Based on the characteristics of respondents, the risk factors for TB HIV co-infection showed that the majority of respondents were at risk of having sex, 24 people (85%) and 3 people (7.5%) having risk factors for drugs. Unsafe sexual behavior such as unprotected sex, unsafe sexual behavior can increase the risk of HIV TB infection (Djalilah & Subagyo, 2021). These risk factors include sex with high-risk partners who show a greater risk of TB-HIV co-infection (RP=25.50; 95% CI) and unsafe behavior in heterosexuals, homosexuals and the use of non-sterile syringes in IDUs (Cahyati, 2019). Drugs are a significant risk factor in the occurrence of HIV-TB coinfection. Drug use can reduce the immune system which can make sufferers more susceptible to TB infection (Andri et al., 2013). Previous studies show that people with HIV co-infected with TB have 6-7 times more risk than those with HIV without TB, which causes the progression of HIV to AIDS more quickly (Permitasari, 2012).

The results of bivariate analysis using the Spearman rank test showed that there was a statistically significant relationship (p value 0.003) between HIV stage and the incidence of tuberculosis. HIV patients in the final stages (stages III to IV) had the highest frequency, 17 people (77.3) experienced TB. This is proven by a similar study in Khartoum State, Sudan which shows that HIV patients with stages III and IV are one of the strong factors that determine the occurrence of tuberculosis infection in HIV patients. In this study, it was explained that HIV patients who have a high stage can increase the odds, thereby increasing the opportunistic infection of tuberculosis (Pradipta et al., 2015). Another study with a retrospective cohort study design at Arba Minch General Hospital, Ethiopia showed that HIV patients in stages III and IV had 20-22 times higher odds of tuberculosis compared to stage I HIV patients (Dalbo & Tamiso, 2016). Tuberculosis infection in HIV patients is closely related to a decrease in the immune system. As the stage of HIV increases, the body's immune system will decrease, which can increase the risk of opportunistic tuberculosis infection (Ekwaru et al., 2013). Tuberculosis and HIV have a strong relationship because HIV infection causes tuberculosis cases to increase and vice versa (Wijaya, 2013). HIV infection is a risk factor for the development of TB through mechanisms in the form of reactivation of latent infections, progression of the primary infection or reinfection with mycobacterium tuberculosis, which will increase tuberculosis cases in the community. Generally, symptoms of pulmonary tuberculosis in HIV sufferers are asymptomatic if the CD4 count is > 350 cells/mL. However, if the CD4 count falls to 50 cells/ μ L, it will be accompanied by extrapulmonary TB (Fauzia, Mahmuda, 2020).

Most people infected with TB germs do not become sick with TB because they have a good immune system. This condition is known as latent TB infection. The relationship between these two diseases is related to the body's immune system which is responsible for fighting infection. HIV is a virus that can weaken the body, which is why other infections can enter the body easily, including TB. In people with a weakened immune system, such as HIV/AIDS patients, latent TB infection can easily develop into active TB. About 10% of people who are not infected with HIV/AIDS, if infected with TB germs, will become sick with TB throughout their life. Meanwhile, in patients with HIV/AIDS who are infected with TB germs, around 60% will become sick with active TB. The risk of progressing from latent TB to active TB is estimated to be between 12-20 times greater in people living with HIV than in those not infected with HIV (Rafael & Nabire, 2021).

The condition of TB co-infection in HIV/AIDS sufferers is the cause of the increasing death rate in HIV/AIDS (around 40-50%). The high mortality rate is mostly found in patients with extrapulmonary TB and smear negative pulmonary TB. This is most likely due to delays in diagnosis and administration of TB therapy. The best way to prevent opportunistic infections is to avoid exposing HIV-infected people to TB. This is rather difficult because TB is an endemic disease in Indonesia. Early detection of TB infection in PLHIV is by carrying out a tuberculin skin test (TST) using purified protein derivative (PPD) 5-TU with the Mantoux (AI) method when the person is diagnosed with HIV. If the TST result is positive, a chest x-ray must be performed immediately and treatment for active TB initiated (Roselinda & Setiawaty, 2016).

CONCLUSION

There is a statistically significant relationship between HIV stage and the incidence of tuberculosis. HIV patients who experience late stages (stages III to IV) have a greater frequency than those experiencing early stages. Tuberculosis infection in HIV patients is closely related to a decrease in the immune system. As the stage of HIV increases, the body's immune system will decrease, so this can increase the risk of opportunistic infection with tuberculosis. The best way to prevent opportunistic infections is to avoid exposing HIV-infected people to tuberculosis.

REFERENCES

- Abdallah, T. M., & Ali, A. A. A. (2012). Epidemiology of tuberculosis in Eastern Sudan. *Asian Pacific Journal of Tropical Biomedicine*, 2(12), 999–1001. [https://doi.org/10.1016/S2221-1691\(13\)60013-1](https://doi.org/10.1016/S2221-1691(13)60013-1)
- Andri, A. J., Poerwandar, E. K., & Bintari, D. R. (2013). Understanding Drug Abusers with HIV/AIDS through Qualitative Research. *Makara Human Behavior Studies in Asia*, 17(1), 64. <https://doi.org/10.7454/mssh.v17i1.1803>
- Bates, M. N., Khalakdina, A., Pai, M., Chang, L., Lessa, F., & Smith, K. R. (2007). Risk of tuberculosis from exposure to tobacco smoke: A systematic review and meta-analysis. *Archives of Internal Medicine*, 167(4), 335–342. <https://doi.org/10.1001/archinte.167.4.335>
- Cahyati, W. H. (2019). Determinan Kejadian Tuberkulosis pada Orang dengan HIV/AIDS. 3(2), 168–178.

- Dalbo, M., & Tamiso, A. (2016). Incidence and Predictors of Tuberculosis among HIV/AIDS Infected Patients: A Five-Year Retrospective Follow-Up Study. *Advances in Infectious Diseases*, 06(02), 70–81. <https://doi.org/10.4236/aid.2016.62010>
- Diedrich, C. R., & Flynn, J. L. (2011). MINIREVIEW HIV-1 / *Mycobacterium tuberculosis* Coinfection Immunology : How Does HIV-1 Exacerbate Tuberculosis? *Immunology*, 79(4), 1407–1417. <https://doi.org/10.1128/IAI.01126-10>
- Djalilah, G. N., & Subagyo, R. (2021). Hubungan Perilaku Seksual Berisiko dengan Kejadian HIV / AIDS Pada Laki Seks Laki The Relationship between Risky Sexual Behavior With the Incidence Of HIV / AIDS Among Men Who Have Sex With Men. 2(1).
- Ekwaru, J. P., Campbell, J., Malamba, S., Moore, D. M., Were, W., & Mermin, J. (2013). The effect of opportunistic illness on HIV RNA viral load and CD4+ T cell count among HIV-positive adults taking antiretroviral therapy. *Journal of the International AIDS Society*, 16(May 2003), 1–6. <https://doi.org/10.7448/IAS.16.1.17355>
- Fauzia, Mahmuda, G. (2020). Hasil Pemeriksaan Kadar Cd4 Dan Tcm Genexpert Mtb. 6(2), 144–150.
- Framasari, D. A., Flora, R., & Sitorus, R. J. (2020). Infeksi Oportunistik pada ODHA Terhadap Kepatuhan Minum ARV di Kota Palembang. *JAMBI MEDICAL JOURNAL “Jurnal Kedokteran Dan Kesehatan,”* 8(1), 67–74.
- Kedzierska, K., Crowe, S. M., Turville, S., & Cunningham, A. L. (2003). The influence of cytokines, chemokines and their receptors on HIV-1 replication in monocytes and macrophages. *Reviews in Medical Virology*, 13(1), 39–56. <https://doi.org/10.1002/rmv.369>
- Kemenkes, Ri. (2017). Data dan Informasi Kesehatan Indonesia 2016.
- Kemenkes, Ri. (2018). Profil Kesehatan Indonesia 2016.
- Liebert, M. A., Coreceptors, U., Rosas-taraco, A. G., Arce-mendoza, A. Y., Caballero-olín, G., & Salinas-carmona, M. C. (2006). *Mycobacterium tuberculosis*. 22(1), 45–51.
- Marfidhotul Iftitah, N., Adi, S., & Warih Gayatri, R. (2020). Faktor Yang Mempengaruhi Terjadinya Ko-Infeksi Tuberculosis Pada Pasien Hiv/Aids Di Kabupaten Malang. *Preventia: Indonesian Journal of Public Health*, 5(1), 27–34.
- M., Corbett, L., Getahun, H., Kittikraisak, W., Heilig, C. M., Corbett, E. L., Ayles, H., Cain, K. P., Grant, A. D., Churchyard, G. J., Kimerling, M., Shah, S., Stephen, D., Wood, R., Maartens, G., Granich, R., Date, A. A., & Varma, J. K. (2011). Development of a Standardized Screening Rule for Tuberculosis in People Living with HIV in Resource-Constrained Settings : Individual Participant Data Development of a Standardized Screening Rule for Tuberculosis in People Living with HIV in Resource- Con. January. <https://doi.org/10.1371/journal.pmed.1000391>
- Patel, N. R., Zhu, J., Tachado, S. D., Zhang, J., Wan, Z., Saukkonen, J., Koziel, H., & Elisa, N.-B. (2007). HIV Impairs TNF- Mediated Macrophage Apoptotic Response to *Mycobacterium tuberculosis*.

- Permitasari, D. A. (2012). Tuberkulosis Pada Pasien Hiv / Aids Di Rsup Dr . Kariadi Semarang Jurnal Media Medika Muda.
- Pradipta, A., Korib Sudaryo, M., Rusli, A., Studi Kesehatan Masyarakat, P., Kesehatan Masyarakat Universitas Indonesia, F., Sulianti Saroso, R., Kesehatan Republik Indonesia, K., & Penulis, K. (2015). Faktor-Faktor yang Berhubungan dengan Infeksi Oportunistik Tuberkulosis pada Pasien HIV di RSPI Sulianti Saroso Tahun. *The Indonesian Journal of Infectious Disease*, 6(2), 18–30.
- Pustil, R. L. (2017). Global AIDS. *AIDS* (London, England), 17 Suppl 4. <https://doi.org/10.1097/00002030-199001001-00040>
- Rafael, S., & Nabire, K. (2021). Profil Pasien HIV / AIDS dengan Koinfeksi Tuberkulosis di Puskesmas Bumi Wonorejo dan Klinik. 31(4), 247–252.
- Roselinda, R., & Setiawaty, V. (2016). The stages of HIV infection and the risk of opportunistic Tuberculosis infection. *Health Science Journal of Indonesia*, 6(2), 121–125. <https://doi.org/10.22435/hsji.v6i2.4771.121-125>
- Rottenberg, M. E., Pawlowski, A., Jansson, M., & Sko, M. (2012). Tuberculosis and HIV Co-Infection. 8(2). <https://doi.org/10.1371/journal.ppat.1002464>
- Tornheim, J. A., & Dooley, K. E. (2017). Tuberculosis Associated with HIV Infection. <https://doi.org/10.1128/microbiolspec.TNMI7-0028-2016>.Correspondence
- WHO. (2017). Global Tuberculosis Reposrt 2017.
- Wijaya, I. M. K. (2013). Infeksi Hiv (Human Immunodeficiency Virus) Pada Penderita Tuberkulosis. Seminar Nasional FMIPA UNDIKSHA III, 3, 295–303. <http://ejournal.undiksha.ac.id/index.php/semnasmipa/article/view/2721/2301>
- Winter, J. R., Smith, C. J., Davidson, J. A., Lalor, M. K., Delpech, V., Abubakar, I., & Stagg, H. R. (2020). The impact of HIV infection on tuberculosis transmission in a country with low tuberculosis incidence: a national retrospective study using molecular epidemiology. 1–15.